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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

INVENTOR(S)		
Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
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☐ *Additional Inventors are being named on the____ separately numbered sheets attached hereto*

TITLE OF THE INVENTION (280 characters max)

CYTOTOXIC INDENO- AND ISOINDOLOISOQUINOLINE COMPOUNDS

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ENCLOSED APPLICATION PARTS *(check all that apply)*

☒ Specification *Number of Pages*	66	☐ CD(s), Number	
☒ Drawing(s) *Number of Sheets*	3	☐ Other (specify)	
☐ Application Data Sheet. See 37 CFR 1.76			

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT *(check one)*

		FILING FEE AMOUNT ($)
☒	Applicant claims small entity status. See 37 CFR 1.27.	
☐	A check or money order is enclosed to cover the filing fees	
☒	The Commissioner is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number: 10-0435	$80.00
☐	Payment by credit card. Form PTO-2038 is attached.	

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☐ No.

☒ Yes, the name of the U.S. Government agency and the Government contract number are: NIH, Grant UO1 CA89566; ST32CA09634, NCI NO1-CO56000; R43 CA79439-01; R44 CA79439-02; R43-CA82964-01

Respectfully submitted,

SIGNATURE

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Date 5/12/2003

REGISTRATION NO. *(if appropriate)* 48,351

Docket Number: 3220-72763

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

This collection of information is required by 37 CFR 1.51. The information is used by the public to file (and by the PTO to process) a provisional application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 8 hours to complete, including gathering, preparing, and submitting the complete provisional application to the PTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, Washington, D.C. 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Box Provisional Application, Assistant Commissioner for Patents, Washington, D.C.

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PATENT APPLICATION

of

Mark S. Cushman

&

Yves G. Pommier

for

Cytotoxic Indeno- and Isoindoloisoquinoline Compounds

Client Reference P-03012.P1.US

Attorney Docket 3220-72763

CYTOTOXIC INDENO- AND ISOINDOLOISOQUINOLINE COMPOUNDS

The government may have rights in this invention. Funding for disclosure was provided in part by the National Institutes of Health (NIH) Research Grant UO1 CA89566 and Training Grant ST32CA09634, the Developmental Therapeutics Program, DCTD, NCI under Contract NO1-CO-56000, and SBIR grants: NCI SBIR Phase 1 Grant # R43 CA79439-01, Anti-Cancer Drug Design Targeting Human Topoisomerase I; NCI SBIR Phase 2 Grant # R44 CA79439-02, Anti-Cancer Drug Design Targeting Human Topoisomerase I, and NCI SBIR Phase 1 Grant # R43-CA82964-01, Anti-Cancer Compounds Designed to Poison Topoisomerase I.

FIELD

This invention relates to indeno- and isoindoloisoquinolines and their use as cytotoxic agents.

BACKGROUND

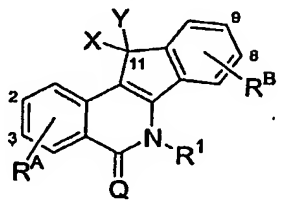
The cytotoxicity profile of the topoisomerase I (top1) inhibitors camptothecin (3) and indenoisoquinoline 2 has been described in Kohlhagen et al. "Protein-Linked DNA Strand Breaks Induced by NSC 314622, a Novel Noncamptothecin Topoisomerase I Poison," *Mol. Pharmacol., 54*, 50-58 (1998); Pommier et al., "Mechanism of Action of Eukaryotic DNA Topoisomerases and Drugs Targeted to the Enzyme," *Biochem. Biophys. Acta, 1400*, 83-105 (1998); and Pommier, "Topoisomerase Inhibitors: Why Develop New Ones," *Opinion in Oncologic, Endocrine & Metabolic Investigational Drugs, 1*, 168-169 (1999), the disclosures of which are incorporated herein by reference (Figure 3). Subsequently, it was discovered that the indenoisoquinoline 2 inhibited top1, and its ability to stabilize the *cleavable complexes* by inhibition of the DNA religation reactions after top1-catalyzed single strand breakage. This inhibition classified 2 as more consistent with a top1 poison than a top1 suppressor. However, the DNA single-strand breaks induced by the indenoisoquinoline 2 were more stable than those induced by

camptothecin (3), and the cleavage site specificity of 2 was different from that of camptothecin (3). The synthesis of indenoisoquinoline 2 is accomplished by treatment of the cis-substituted isoquinolone 1 with thionyl chloride, as described in Cushman & Cheng, "Stereoselective Oxidation by Thionyl Chloride Leading to the Indeno[1,2-*c*]isoquinoline System," *J. Org. Chem. 43*, 3781-83 (1978), the disclosure of which is incorporated herein by reference (Figure 3).

Although several camptothecin (3) derivatives such as irinotecan and topotecan are clinically useful anticancer agents, they are unstable due to lactone ring opening, and subsequently rapid reversibility of the cleavage complexes after drug removal is observed. Consequently, there is a present need for additional therapeutic agents that inhibit top1 like the camptothecins, but that induce novel DNA cleavage patterns, have modified toxicity profiles and extended durations of action, and display different antitumor spectra relative to the camptothecins themselves. A number of analogues of the indenoisoquinoline 2 have been synthesized, as described in Strumberg et al., "Synthesis of Cytotoxic Indenoisoquinoline Topoisomerase I Poisons," *J. Med. Chem.*, *42*, 446-457 (1999); Cushman et al., "Synthesis of New Indeno[1,2-*c*]isoquinolines: Cytotoxic Non-Camptothecin Topoisomerase I Inhibitors. *J. Med. Chem.*, *43*, 3688-3698 (2000); Jayaraman et al., "Synthesis of New Dihydroindeno[1,2-*c*]isoquinoline and Indenoisoquinolinium Chloride Topoisomerase I Inhibitors Having High in Vivo Anticancer Activity in the Hollow Fiber Animal Model," *J. Med. Chem.*, *45*, 242-249 (2002).

SUMMARY OF THE INVENTION

A compound having the formula **I**:



I

where Q is oxygen or sulfur;

X is hydrogen and Y is CR^2R^3, or X and Y are taken together to form $=CR^2R^3$; $=NR^2$; $=NOR^2$; or $=NNR^2R^3$;

R^1, R^2, and R^3 are each independently selected from the group consisting of hydrogen and a radical $-(CH_2)_mZ$, where m is 0-6 and Z is selected from the group consisting of halogen, hydroxy, formyl, C_1-C_6 alkanoyloxy, optionally-substituted benzoyloxy, C_1-C_6 alkyl, C_1-C_6 alkoxy, C_3-C_8 cycloalkyl, C_3-C_8 cycloalkoxy, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_1-C_6 haloalkoxy, C_3-C_8 halocycloalkyl, C_3-C_8 halocycloalkoxy, amino, C_1-C_6 alkylamino, (C_1-C_6 alkyl)(C_1-C_6 alkyl)amino, amido, N-(C_1-C_6 alkyl)amido, cyano, nitro, C_1-C_6 alkylsulfonyl, optionally-substituted phenyl, and optionally-substituted phenoxy; or Z is selected from the group consisting of $-CO_2R^4$ and $-CONR^5R^6$, where R^4, R^5, and R^6 are each independently selected from the group consisting of hydrogen, C_1-C_6 alkyl, C_3-C_8 cycloalkyl, C_1-C_6 haloalkyl, optionally-substituted phenyl, and optionally-substituted phenyl-C_1-C_6 alkyl; or

when X and Y are taken together to form $=NNR^2R^3$, R^2 and R^3 are taken together with the attached nitrogen to form an optionally-substituted heterocycle;

R^A represents 1-4 substituents each independently selected from the group consisting of hydrogen and a radical $-(CH_2)_{m'}Z'$, where m′ is 0-6 and Z′ is selected from the group consisting of halogen, hydroxy, C_1-C_6 alkanoyloxy, optionally-substituted benzoyloxy, C_1-C_6 alkyl, C_1-C_6 alkoxy, C_3-C_8 cycloalkyl, C_3-C_8 cycloalkoxy, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_1-C_6 haloalkoxy, C_3-C_8 halocycloalkyl, C_3-C_8 halocycloalkoxy, amino, C_1-C_6 alkylamino, (C_1-C_6 alkyl)(C_1-C_6 alkyl)amino, amido, N-(C_1-C_6 alkyl)amido, cyano, nitro, C_1-C_6 alkylsulfonyl, optionally-substituted phenyl, and optionally-substituted phenoxy; or Z′ is selected from the group consisting of $-CO_2R^{4'}$ and $-CONR^{5'}R^{6'}$, where $R^{4'}$, $R^{5'}$, and $R^{6'}$ are each independently selected from the group consisting of hydrogen, C_1-C_6 alkyl, C_3-C_8 cycloalkyl, C_1-C_6 haloalkyl, optionally-substituted phenyl, and optionally-substituted phenyl-C_1-C_6 alkyl; or

R^A represents 2-4 substituents where 2 of said substituents are adjacent substituents and are taken together with the attached carbons to form an optionally-substituted carbocycle or an optionally-substituted heterocycle, and the remaining 2

substituents are each independently selected from the group consisting of hydrogen and a radical -$(CH_2)_{m'}Z'$, where m' is 0-6 and Z' is selected from the group consisting of hydrogen, halogen, hydroxy, C_1-C_6 alkanoyloxy, optionally-substituted benzoyloxy, C_1-C_6 alkyl, C_1-C_6 alkoxy, C_3-C_8 cycloalkyl, C_3-C_8 cycloalkoxy, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_1-C_6 haloalkoxy, C_3-C_8 halocycloalkyl, C_3-C_8 halocycloalkoxy, amino, C_1-C_6 alkylamino, (C_1-C_6 alkyl)(C_1-C_6 alkyl)amino, amido, N-(C_1-C_6 alkyl)amido, cyano, nitro, C_1-C_6 alkylsulfonyl, optionally-substituted phenyl, and optionally-substituted phenoxy; or Z' is selected from the group consisting of -$CO_2R^{4'}$ and -$CONR^{5'}R^{6'}$, where $R^{4'}$, $R^{5'}$, and $R^{6'}$ are each independently selected from the group consisting of hydrogen, C_1-C_6 alkyl, C_3-C_8 cycloalkyl, C_1-C_6 haloalkyl, optionally-substituted phenyl, and optionally-substituted phenyl-C_1-C_6 alkyl; and

R^B represents 1-4 substituents each independently selected from the group consisting of hydrogen and a radical -$(CH_2)_{m''}Z''$, where m'' is 0-6 and Z'' is selected from the group consisting of halogen, hydroxy, C_1-C_6 alkanoyloxy, optionally-substituted benzoyloxy, C_1-C_6 alkyl, C_1-C_6 alkoxy, C_3-C_8 cycloalkyl, C_3-C_8 cycloalkoxy, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_1-C_6 haloalkoxy, C_3-C_8 halocycloalkyl, C_3-C_8 halocycloalkoxy, amino, C_1-C_6 alkylamino, (C_1-C_6 alkyl)(C_1-C_6 alkyl)amino, amido, N-(C_1-C_6 alkyl)amido, cyano, nitro, C_1-C_6 alkylsulfonyl, optionally-substituted phenyl, and optionally-substituted phenoxy; or Z'' is selected from the group consisting of -$CO_2R^{4''}$ and -$CONR^{5''}R^{6''}$, where $R^{4''}$, $R^{5''}$, and $R^{6''}$ are each independently selected from the group consisting of hydrogen, C_1-C_6 alkyl, C_3-C_8 cycloalkyl, C_1-C_6 haloalkyl, optionally-substituted phenyl, and optionally-substituted phenyl-C_1-C_6 alkyl; or

R^B represents 2-4 substituents where 2 of said substituents are adjacent substituents and are taken together with the attached carbons to form an optionally-substituted carbocycle or an optionally-substituted heterocycle, and the remaining 2 substituents are each independently selected from the group consisting of hydrogen and a radical -$(CH_2)_{m''}Z''$, where m'' is 0-6 and Z'' is selected from the group consisting of hydrogen, halogen, hydroxy, C_1-C_6 alkanoyloxy, optionally-substituted benzoyloxy, C_1-C_6 alkyl, C_1-C_6 alkoxy, C_3-C_8 cycloalkyl, C_3-C_8 cycloalkoxy, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_1-C_6 haloalkoxy, C_3-C_8 halocycloalkyl, C_3-

C_8 halocycloalkoxy, amino, C_1-C_6 alkylamino, (C_1-C_6 alkyl)(C_1-C_6 alkyl)amino, amido, N-(C_1-C_6 alkyl)amido, cyano, nitro, C_1-C_6 alkylsulfonyl, optionally-substituted phenyl, and optionally-substituted phenoxy; or Z″ is selected from the group consisting of -$CO_2R^{4''}$ and -$CONR^{5''}R^{6''}$, where $R^{4''}$, $R^{5''}$, and $R^{6''}$ are each independently selected from the group consisting of hydrogen, C_1-C_6 alkyl, C_3-C_8 cycloalkyl, C_1-C_6 haloalkyl, optionally-substituted phenyl, and optionally-substituted phenyl-C_1-C_6 alkyl is described.

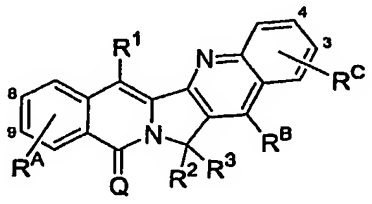
In one illustrative embodiment, Q is oxygen, R^A is 2,3-bis(C_1-C_6 alkoxy), R^B is 8,9-alkylenedioxy, and X and Y are taken together to form $=CR^2R^3$, where R^2 is hydrogen. In another illustrative embodiment, Q is oxygen, R^A is 2,3-bis(C_1-C_6 alkoxy), R^B is 8,9-alkylenedioxy, X and Y are taken together to form $=CR^2R^3$, R^2 is hydrogen, and R^1 is hydrogen, C_1-C_6 alkyl, C_3-C_8 cycloalkyl, C_1-C_6 haloalkyl, C_3-C_8 halocycloalkyl, amino-C_1-C_6 alkyl, C_1-C_6 alkylamino-C_1-C_6 alkyl, or (C_1-C_6 alkyl)(C_1-C_6 alkyl)amino-C_1-C_6 alkyl.

In some embodiments, when X and Y are taken together to form a double bond, the double bond geometry is illustratively E, or entgegen.

A pharmaceutical composition including a compound having the formula **I** and a pharmaceutically acceptable carrier, excipient, or diluent therefore is also described.

A method for treating a mammal in need of relief from a disease state including cancer, comprising administering to the mammal an effective amount of a compound having the formula **I** or of a pharmaceutical composition including a compound having the formula **I** is also described.

A compound having the formula **II**:



II

where Q is oxygen or sulfur;

R^1, R^2, and R^3 are each independently selected from the group consisting of hydrogen and a radical $-(CH_2)_mZ$, where m is 0-6 and Z is selected from the group consisting of halogen, hydroxy, formyl, C_1-C_6 alkanoyloxy, optionally-substituted benzoyloxy, C_1-C_6 alkyl, C_1-C_6 alkoxy, C_3-C_8 cycloalkyl, C_3-C_8 cycloalkoxy, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_1-C_6 haloalkoxy, C_3-C_8 halocycloalkyl, C_3-C_8 halocycloalkoxy, amino, C_1-C_6 alkylamino, (C_1-C_6 alkyl)(C_1-C_6 alkyl)amino, amido, N-(C_1-C_6 alkyl)amido, cyano, nitro, C_1-C_6 alkylsulfonyl, optionally-substituted phenyl, and optionally-substituted phenoxy; or Z is selected from the group consisting of $-CO_2R^4$ and $-CONR^5R^6$, where R^4, R^5, and R^6 are each independently selected from the group consisting of hydrogen, C_1-C_6 alkyl, C_3-C_8 cycloalkyl, C_1-C_6 haloalkyl, optionally-substituted phenyl, and optionally-substituted phenyl-C_1-C_6 alkyl; or

R^1 is selected from the group consisting of hydrogen and a radical $-(CH_2)_mZ$, where m is 0-6 and Z is selected from the group consisting of halogen, hydroxy, formyl, C_1-C_6 alkanoyloxy, optionally-substituted benzoyloxy, C_1-C_6 alkyl, C_1-C_6 alkoxy, C_3-C_8 cycloalkyl, C_3-C_8 cycloalkoxy, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_1-C_6 haloalkoxy, C_3-C_8 halocycloalkyl, C_3-C_8 halocycloalkoxy, amino, C_1-C_6 alkylamino, (C_1-C_6 alkyl)(C_1-C_6 alkyl)amino, amido, N-(C_1-C_6 alkyl)amido, cyano, nitro, C_1-C_6 alkylsulfonyl, optionally-substituted phenyl, and optionally-substituted phenoxy; or Z is selected from the group consisting of $-CO_2R^4$ and $-CONR^5R^6$, where R^4, R^5, and R^6 are each independently selected from the group consisting of hydrogen, C_1-C_6 alkyl, C_3-C_8 cycloalkyl, C_1-C_6 haloalkyl, optionally-substituted phenyl, and optionally-substituted phenyl-C_1-C_6 alkyl; and R^2 and R^3 are taken together with the attached carbon to form an optionally-substituted carbocycle or heterocycle;

R^A represents 1-4 substituents each independently selected from the group consisting of hydrogen and a radical $-(CH_2)_{m'}Z'$, where m′ is 0-6 and Z′ is selected from the group consisting of halogen, hydroxy, C_1-C_6 alkanoyloxy, optionally-substituted benzoyloxy, C_1-C_6 alkyl, C_1-C_6 alkoxy, C_3-C_8 cycloalkyl, C_3-C_8 cycloalkoxy, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_1-C_6 haloalkoxy, C_3-C_8 halocycloalkyl, C_3-C_8 halocycloalkoxy, amino, C_1-C_6 alkylamino, (C_1-C_6 alkyl)(C_1-C_6 alkyl)amino, amido, N-(C_1-C_6 alkyl)amido, cyano, nitro, C_1-C_6 alkylsulfonyl,

optionally-substituted phenyl, and optionally-substituted phenoxy; or Z′ is selected from the group consisting of $-CO_2R^{4'}$ and $-CONR^{5'}R^{6'}$, where $R^{4'}$, $R^{5'}$, and $R^{6'}$ are each independently selected from the group consisting of hydrogen, C_1-C_6 alkyl, C_3-C_8 cycloalkyl, C_1-C_6 haloalkyl, optionally-substituted phenyl, and optionally-substituted phenyl-C_1-C_6 alkyl; or

R^A represents 2-4 substituents where 2 of said substituents are adjacent substituents and are taken together with the attached carbons to form an optionally-substituted carbocycle or an optionally-substituted heterocycle, and the remaining 2 substituents are each independently selected from the group consisting of hydrogen and a radical $-(CH_2)_{m'}Z'$, where m′ is 0-6 and Z′ is selected from the group consisting of hydrogen, halogen, hydroxy, C_1-C_6 alkanoyloxy, optionally-substituted benzoyloxy, C_1-C_6 alkyl, C_1-C_6 alkoxy, C_3-C_8 cycloalkyl, C_3-C_8 cycloalkoxy, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_1-C_6 haloalkoxy, C_3-C_8 halocycloalkyl, C_3-C_8 halocycloalkoxy, amino, C_1-C_6 alkylamino, (C_1-C_6 alkyl)(C_1-C_6 alkyl)amino, amido, N-(C_1-C_6 alkyl)amido, cyano, nitro, C_1-C_6 alkylsulfonyl, optionally-substituted phenyl, and optionally-substituted phenoxy; or Z′ is selected from the group consisting of $-CO_2R^{4'}$ and $-CONR^{5'}R^{6'}$, where $R^{4'}$, $R^{5'}$, and $R^{6'}$ are each independently selected from the group consisting of hydrogen, C_1-C_6 alkyl, C_3-C_8 cycloalkyl, C_1-C_6 haloalkyl, optionally-substituted phenyl, and optionally-substituted phenyl-C_1-C_6 alkyl;

R^B is selected from the group consisting of hydrogen and a radical $-(CH_2)_{m''}Z''$, where m″ is 0-6 and Z″ is selected from the group consisting of halogen, hydroxy, C_1-C_6 alkanoyloxy, optionally-substituted benzoyloxy, C_1-C_6 alkyl, C_1-C_6 alkoxy, C_3-C_8 cycloalkyl, C_3-C_8 cycloalkoxy, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_1-C_6 haloalkoxy, C_3-C_8 halocycloalkyl, C_3-C_8 halocycloalkoxy, amino, C_1-C_6 alkylamino, (C_1-C_6 alkyl)(C_1-C_6 alkyl)amino, amido, N-(C_1-C_6 alkyl)amido, cyano, nitro, C_1-C_6 alkylsulfonyl, optionally-substituted phenyl, and optionally-substituted phenoxy; or Z″ is selected from the group consisting of $-CO_2R^{4''}$ and $-CONR^{5''}R^{6''}$, where $R^{4''}$, $R^{5''}$, and $R^{6''}$ are each independently selected from the group consisting of hydrogen, C_1-C_6 alkyl, C_3-C_8 cycloalkyl, C_1-C_6 haloalkyl, optionally-substituted phenyl, and optionally-substituted phenyl-C_1-C_6 alkyl; and′

R^C represents 1-4 substituents each independently selected from the group consisting of hydrogen and a radical $-(CH_2)_{m'''}Z'''$, where m''' is 0-6 and Z''' is selected from the group consisting of halogen, hydroxy, C_1-C_6 alkanoyloxy, optionally-substituted benzoyloxy, C_1-C_6 alkyl, C_1-C_6 alkoxy, C_3-C_8 cycloalkyl, C_3-C_8 cycloalkoxy, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_1-C_6 haloalkoxy, C_3-C_8 halocycloalkyl, C_3-C_8 halocycloalkoxy, amino, C_1-C_6 alkylamino, (C_1-C_6 alkyl)(C_1-C_6 alkyl)amino, amido, N-(C_1-C_6 alkyl)amido, cyano, nitro, C_1-C_6 alkylsulfonyl, optionally-substituted phenyl, and optionally-substituted phenoxy; or Z'' is selected from the group consisting of $-CO_2R^{4'''}$ and $-CONR^{5'''}R^{6'''}$, where $R^{4'''}$, $R^{5'''}$, and $R^{6'''}$ are each independently selected from the group consisting of hydrogen, C_1-C_6 alkyl, C_3-C_8 cycloalkyl, C_1-C_6 haloalkyl, optionally-substituted phenyl, and optionally-substituted phenyl-C_1-C_6 alkyl; or

R^C represents 2-4 substituents where 2 of said substituents are adjacent substituents and are taken together with the attached carbons to form an optionally-substituted carbocycle or an optionally-substituted heterocycle, and the remaining 2 substituents are each independently selected from the group consisting of hydrogen and a radical $-(CH_2)_{m'''}Z'''$, where m''' is 0-6 and Z''' is selected from the group consisting of hydrogen, halogen, hydroxy, C_1-C_6 alkanoyloxy, optionally-substituted benzoyloxy, C_1-C_6 alkyl, C_1-C_6 alkoxy, C_3-C_8 cycloalkyl, C_3-C_8 cycloalkoxy, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_1-C_6 haloalkoxy, C_3-C_8 halocycloalkyl, C_3-C_8 halocycloalkoxy, amino, C_1-C_6 alkylamino, (C_1-C_6 alkyl)(C_1-C_6 alkyl)amino, amido, N-(C_1-C_6 alkyl)amido, cyano, nitro, C_1-C_6 alkylsulfonyl, optionally-substituted phenyl, and optionally-substituted phenoxy; or Z''' is selected from the group consisting of $-CO_2R^{4'''}$ and $-CONR^{5'''}R^{6'''}$, where $R^{4'''}$, $R^{5'''}$, and $R^{6'''}$ are each independently selected from the group consisting of hydrogen, C_1-C_6 alkyl, C_3-C_8 cycloalkyl, C_1-C_6 haloalkyl, optionally-substituted phenyl, and optionally-substituted phenyl-C_1-C_6 alkyl is described.

In one illustrative embodiment, Q is oxygen, R^A is 2,3-bis(C_1-C_6 alkoxy), and R^B, R^C, R^1, $R^2$and R^3 are each hydrogen.

A pharmaceutical composition comprising a compound having the formula II and a pharmaceutically acceptable carrier, excipient, or diluent therefore is also described.

A method for treating a mammal in need of relief from a disease state including cancer, comprising administering to the mammal an effective amount of a compound having the formula II or of a pharmaceutical composition including a compound having the formula II is also described.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 show a ^{1}H NMR Spectrum of indenoisoquinoline **19**.

Figure 2 shows a model of the binding of isoindoloisoquinoline **13** in the ternary complex consisting of DNA, top1, and the inhibitor. The diagram is programmed for wall-eyed viewing.

Figure 3 shows various indenoisoquinolines, isoindoloisoquinolines, and derivatives thereof.

DETAILED DESCRIPTION OF THE INVENTION

In one embodiment, isoindoloisoquinoline compounds are described. In one aspect, the isoindoloisoquinoline compounds are benzoisoindoloisoquinolines. Without being bound by theory, the lactam of the indenoisoquinoline **2** may correspond to the lactam of camptothecin (**3**), and the two methoxyl oxygens of **2** may correspond to the two lactone oxygens of **3**. Illustratively, compounds **8** and **13** are prepared.

In another embodiment, attachment of haloalkenyl and aminoalkenyl side chains to C-11 of compound **2** are described. Prior DNA unwinding studies had indicated that *N*-3-aminoalkyl derivatives of the indenoisoquinoline ring system can intercalate. Without being bound by theory, the cationic side chain on the indenoisoquinoline nitrogen of compound **7** may project into the major groove towards the Asn352 residue of the protein. Similarly, the attachment of alkenyl side chains using the C-11 ketone as a reactive functional group would afford indenoisoquinolines having side chains that would project into the minor groove, toward the Arg364 and Asp533 residues of the enzyme.

The present invention further provides pharmaceutical formulations comprising an effective amount of an indenoisoquinoline or isoindoloisoquinoline compound of this invention for treating a patient having cancer. As used herein, an

effective amount of the indenoisoquinoline or isoindoloisoquinoline compound is defined as the amount of the compound which, upon administration to a patient, inhibits growth of cancer cells, kills malignant cells, reduces the volume or size of the tumors or eliminates the tumor entirely in the treated patient.

The effective amount to be administered to a patient is typically based on body surface area, patient weight, and/or patient condition. The interrelationship of dosages for animals and humans (based on milligrams per meter squared of body surface) is described by Freireich, E.J., et al., *Cancer Chemother. Rep.* 1966, 50 (4), 219. Body surface area may be approximately determined from patient height and weight (see e.g., Scientific Tables, Geigy Pharmaceuticals, Ardley, New York, pages 537-538 (1970)). An effective amount of the indenoisoquinoline or isoindoloisoquinoline compounds of the present invention is defined as any amount useful for inhibiting the growth of (or killing) cancer cells in a patient. Typically, such effective amounts range from about 5 mg/kg to about 500 mg/kg, more preferably from about 5 mg/kg to about 250 mg/kg, and most preferably about 5 to about 150 mg/kg. Effective doses will also vary, as recognized by those skilled in the art, dependent on route of administration, excipient usage, and the possibility of co-usage with other therapeutic treatments including other anti-tumor agents, and radiation therapy.

The pharmaceutical formulation may be administered via the parenteral route, including subcutaneously, intraperitoneally, intramuscularly, and intravenously. Examples of parenteral dosage forms include aqueous solutions of the active agent, in isotonic saline, 5% glucose or other well-known pharmaceutically acceptable liquid carrier. In one preferred aspect of the present embodiment, the indenoisoquinoline or isoindoloisoquinoline compound is dissolved in a saline solution containing 5% dimethyl sulfoxide and 10% Cremphor EL (Sigma Chemical Company). Additional solubilizing agents such as cyclodextrins, which can form specific, more soluble complexes with the present indenoisoquinoline or isoindoloisoquinoline compounds, or other solubilizing agents well-known to those familiar with the art, can be utilized as pharmaceutical excipients for delivery of the indenoisoquinoline or isoindoloisoquinoline compounds.

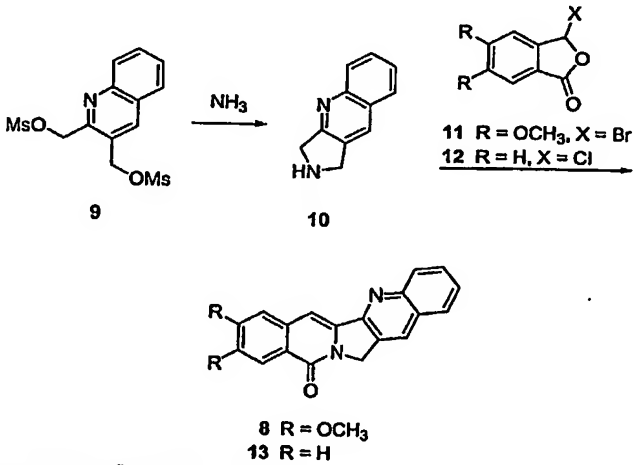
The present compound can also be formulated into dosage forms for other routes of administration utilizing well-known methods. The pharmaceutical compositions can be formulated, for example, in dosage forms for oral administration in a capsule, a gel seal or a tablet. Capsules may comprise any well-known pharmaceutically acceptable material such as gelatin or cellulose derivatives. Tablets may be formulated in accordance with conventional procedure by compressing mixtures of the active indenoisoquinoline or isoindoloisoquinoline and solid carriers, and lubricants well-known to those familiar with the art. Examples of solid carriers include starch, sugar and bentonite. The compounds of the present invention can also be administered in a form of a hard shell tablet or capsule containing, for example, lactose or mannitol as a binder and conventional fillers and tableting agents.

The examples provided illustrate various embodiments of Applicants' invention, and are not intended to in any way limit the scope of the invention as set forth in this specification and claims.

Example 1. General synthesis of compounds II.

Syntheses of the benzoisoindoloisoquinoline **8** and the analog compound **13**, which lacks the two methoxyl groups of **8**, are outlined in Scheme 1.

Scheme 1



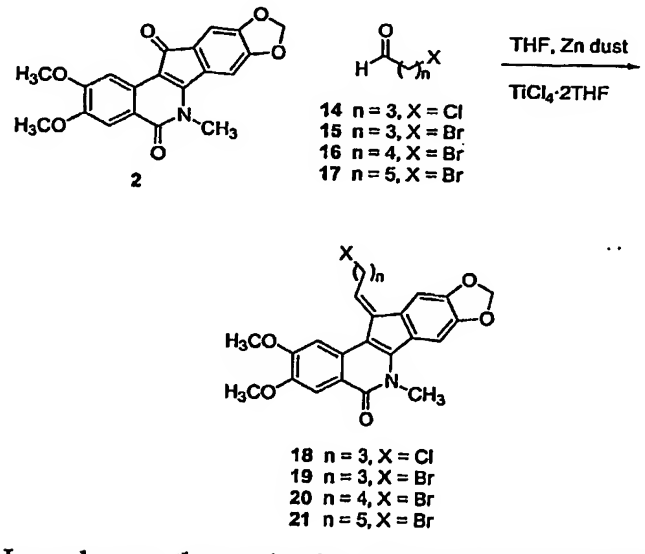
Treatment of a THF solution of the dimesylate **9** with liquid ammonia afforded the 2,3-dihydro-1*H*-pyrrolo[3,4-*b*]quinoline (**10**), which was reacted *in situ* with bromide **11** to afford the desired compound **8**. Detailed descriptions of the reaction conditions are generally described in Corey et al., "Total Synthesis of Natural

20(*S*)-Camptothecin," *J. Org. Chem.*, *40*, 2140-41 (1975); Claus & Steinitz, "Alkyl Derivatives of β-Quinaldic Acid," *Justus Liebigs Ann. Chem.*, *282*, 107-30 (1894); Parrick & Ragunathan, "Studies of Phthalazine-5,8-quinone, A Ring Contraction, and Some Novel and Potentially Useful Fluorescent Phthalimides," *J. Chem. Soc. Perkin Trans. 1*, 211-16 (1993); Slemon et al., "Synthesis of Phthalideisoquinolines from 3-Halopyridines and 3,4-Dihydroisoquinolinium Salts," *Can. J. Chem.*, *59*, 3055-60 (1981), the disclosures of which are incorporated herein by reference. Similarly, reaction of **10** with the chloride **12** yielded the corresponding unsubstituted derivative **13**. Preparation of chloride **12** is generally described in Sloan & Koch, "Effect of Nucleophilicity and Leaving Group Ability on the S_N2 Reactions of Amines with (Acyloxy)alkyl α-Halides." *J. Org. Chem.*, *48*, 635-640 (1983), the disclosure of which is incorporated herein by reference.

Example 2. General synthesis of compounds **I**.

As portrayed in Scheme 2, the 11-indenoisoquinolines **18-21** were prepared using a McMurry reaction of the ketone **2** with the haloaldehydes **14-17**.

Scheme 2



In each case, the production of a single double bond isomer was observed. The stereochemistry of the double bond was determined by obtaining nuclear Overhauser effect (NOE) difference spectra of compound **19**. The interpretation of the NOE difference spectra is based on the assignments of the signals

in the ^{1}H NMR spectrum of **19** (Figure 1). The H_B, H_E, L, F, G, and H protons were assigned to the resonances at 7.89, 6.86, 6.08, 3.00, 2.28, and 3.61 ppm, respectively (see Figure 1). The resonance at 7.89 ppm was assigned to H_B because this proton is adjacent to the amide carbonyl, which would presumably deshield H_B and cause it to shift farthest downfield with respect to the other aromatic protons. The resonance at 6.68 ppm was assigned to the vinylic proton H_E because this is the only triplet that might integrate for one proton. Furthermore, the resonance at 6.08 ppm was assigned to the L protons because they are the only protons that might appear as a two-proton singlet. The assignment of protons at F, G, and H was accomplished by evaluating their chemical shifts. The protons at H are adjacent to a bromide and are farthest downfield at 3.60 ppm. The allylic protons at F are further upfield at 3.00 ppm, and finally the protons at G are farthest upfield at 2.28 ppm.

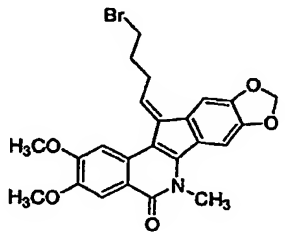
The remaining aromatic resonances were assigned using NOE difference spectrometry. Irradiation of the L protons (6.08 ppm) resulted in enhancement of the resonances at 7.39 and 7.36 ppm, corresponding to H_D and H_C. Because the resonance at 7.89 ppm was assigned to H_B, the resonance at 7.44 ppm should correspond to H_A by the process of elimination. Irradiation of H_E (6.86 ppm) resulted in a strong enhancement of the resonance at 7.44 ppm corresponding to H_A instead of H_D. Therefore, the double bond at C-11 of indenoisoquinoline 4 supports the assigned *E* stereochemistry.

The assignments of the remainder of the resonances in the NMR spectrum of **19** (Figure 1) were accomplished in the same manner and supported the assignments made above. Specifically, irradiation of the F protons (3.00 ppm) caused an enhancement of the resonance at 7.36 ppm. Therefore, the resonance at 7.36 ppm corresponds to H_D. Through the process of elimination, the resonance at 7.39 ppm must belong to H_C. Irradiation of H_C (7.39 ppm) resulted in enhancement of the resonance at 4.040 ppm, resulting in assignment of this resonance to the protons of the methyl amine K. Finally, irradiation of H_B (7.89 ppm) resulted in enhancement of the resonance at 4.023 ppm. Therefore, the resonance at 4.023 ppm corresponds to the protons at J, and by process of elimination, the resonance at 4.044 belongs to the protons at I.

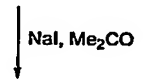
Though as exemplified herein, the McMurray coupling reaction affords only the double bond having the E configuration, it is appreciated that other double bond forming reactions may afford either the Z configuration or a mixture of E and Z double bond isomers.

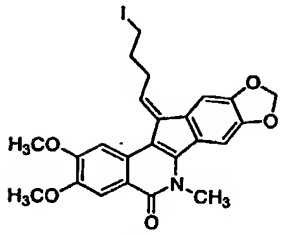
The 4-iodobutenyl compound **24** was derived from the bromide **19** using the Finkelstein reaction (Scheme 3).

Scheme 3



19

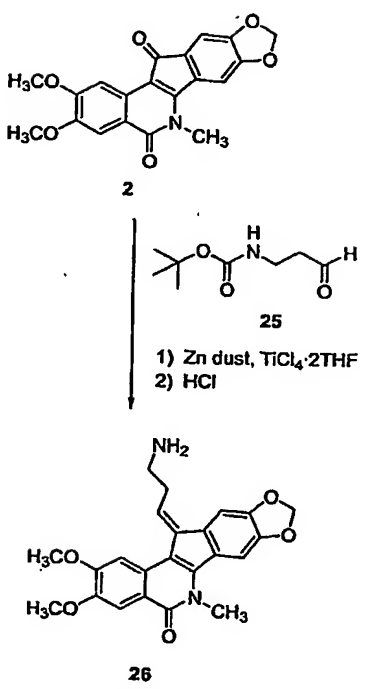




24

As with the haloalkene derivatives, the McMurry reaction of the lead compound **2** with Boc-protected β-alaninal **(25)** afforded the desired 3-aminopropenyl compound **26** after acidic work-up (Scheme 4).

Scheme 4



Alaninal **25** may be prepared as described in Blaney et al., "Fused and Bridged Bi- and Tri-Cyclic Lactams via Sequential Metallo-Azomethine Ylide Cycloaddition-Lactamisation," *Tetrahedron*, *58*, 1719-37 (2002), the disclosure of which is incorporated herein by reference.

Example 3. Biological activity of compounds I.

The indenoisoquinolines were examined for antiproliferative activity against the human cancer cell lines in the National Cancer Institute screen, in which the activity of each compound was evaluated with approximately 55 different cancer cell lines of diverse tumor origins. The GI50 values obtained with selected cell lines, along with the mean graph midpoint (MGM) values, are summarized in Table 1. The MGM is based on a calculation of the average GI50 for all of the cell lines tested (approximately 55) in which GI50 values below and above the test range (10^{-8} to 10^{-4} molar) are taken as the minimum (10^{-8} molar) and maximum (10^{-4} molar) drug concentrations used in the screening test. In addition, the relative activities of the compounds in the top1-mediated DNA cleavage assay are listed in Table 1. For comparison purposes, the activities of the previously reported lead compound **2** and its more potent *N*-3'-aminoalkyl derivative **7** are also included in the table.

Without being bound by theory, a hydrogen bonding interaction between the hydroxyl group of the camptothecin analogue topotecan and the carboxyl group of Asp533 of the enzyme may contribute to the binding of the camptothecin ring system, and an analogous interaction is less likely with **8** and **13**, which may help to explain why these analogues are less potent than camptothecin. In addition, the recently published X-ray crystallography studies show that the carboxylate and hydroxyl groups of the ring-opened hydroxyacid form of the lactone **28** of topotecan (**29**) also contribute to its binding in the ternary complex.

A model was constructed by overlapping the structure of the benzoisoindoloisoquinoline **13** with the structure of topotecan in the published ternary complex and then deleting the camptothecin structure (Figure 2). *See* Staker et al., "The Mechanism of Topoisomerase I Poisoning by a Camptothecin Analog," *Proc. Natl. Acad. Sci. U.S.A.*, *99*, 15387-92 (2002), the disclosure of which is incorporated herein by reference. The synthetic double-stranded DNA in the crystalline ternary complex contained a phosphorothiolate at the cleavage site.

It is appreciated that cytotoxicity profile of compounds described herein may be improved by altering solubility properties, facilitating cellular uptake, and/or the inclusion of components that take advantage of the electrostatic attraction of a positively-charged ammonium cation to a negatively-charged DNA phosphodiester backbone prior to intercalation into the cleavage complex, as illustrated by compounds **26** and **7**.

Example 4. 8,9-Dimethoxy-12*H*-5,11a-diaza-dibenzo[*b,h*]fluoren-11-one (8).

A solution of **9** (described in Claus & Steinitz, "Alkyl Derivatives of β-Quinaldic Acid," *Justus Liebigs Ann. Chem.*, *282*, 107-130 (1894), the disclosure of which is incorporated herein by reference) (200 mg, 0.58 mmol) in anhydrous THF (20 mL) was degassed by bubbling argon through the solution for 30 min. Liquid NH_3 was added via cold finger for 5 min at approximately 1 drop/5sec. The cold finger was removed and the reaction mixture was allowed to warm to room temperature under argon. The reaction mixture was stirred at room temperature for 12 h, at which point argon was bubbled through the solution for 1.5 h to remove excess NH_3. Anhydrous THF (10 mL) and NEt_3 (3 mL) were added and the reaction mixture was stirred at room temperature for 30 min. Bromide **11** (described in Slemon et al.,

"Synthesis of Phthalideisoquinolines from 3-Halopyridines and 3,4-Dihydroisoquinolinium Salts," *Can. J. Chem., 59*, 3055-3060 (1981), the disclosure of which is incorporated herein by reference) was added and the reaction mixture was stirred at room temperature for 24 h. The solvent was removed in vacuo and replaced with 10% NaOAc/AcOH (30 mL). The reaction mixture was stirred at room temperature for 24 h, at which point the solvent was removed in vacuo. The resulting solid was dissolved in water (100 mL) and extracted with $CHCl_3$ (3 × 100 mL). The organic layers were pooled, washed with saturated aqueous $NaHCO_3$ (1 × 100 mL), brine, dried ($MgSO_4$), filtered, and concentrated in vacuo to provide a brown solid. Purification (silica gel, $CHCl_3$) provided **8** (106 mg, 53%) as a yellow solid: ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.61 (s, 1 H), 8.12 (m, 2 H), 7.84 (m, 1 H), 7.71 (s, 1 H), 7.82 (m, 1 H), 7.61 (s, 1 H), 7.50 (s, 1 H), 5.32 (s, 2 H), 3.94 (s, 3 H), 3.91 (s, 3 H); ESIMS *m/z* (rel intensity) 345.2 (100, MH^+). Anal. Calcd for $C_{21}H_{16}N_2O_3 \cdot 0.5H_2O$: C, 71.38; H, 4.85; N, 7.93. Found: C, 71.07; H, 4.72; N, 7.85.

Example 5. 12*H*-5,11a-Diaza-dibenzo[*b,h*]fluoren-11-one (13).

A solution of **9** (200 mg, 0.58 mmol) in anhydrous THF (20 mL) was degassed by bubbling argon through the solution for 30 min. Liquid NH_3 was added via cold finger for 5 min at approximately 1 drop/5sec. The cold finger was removed and the reaction mixture was allowed to warm to room temperature under argon. The reaction mixture was stirred at room temperature for 12 h, at which point argon was bubbled through the solution for 1.5 h to remove excess NH_3, affording a solution of intermediate **10**. Anhydrous THF (10 mL) and NEt_3 (3 mL) were added and the reaction mixture was stirred at room temperature for 30 min. Chloride **12** (Sloan & Koch, "Effect of Nucleophilicity and Leaving Group Ability on the S_N2 Reactions of Amines with (Acyloxy)alkyl α-Halides," *J. Org. Chem., 48*, 635-640 (1983), the disclosure of which is incorporated herein by reference) (195 mg, 1.16 mmol) was added and the reaction mixture stirred at room temperature for 24 h. The solvent was removed in vacuo and replaced with 10% NaOAc/AcOH (30 mL). The reaction mixture was stirred at room temperature for 24 h, at which point the solvent was removed in vacuo. The resulting solid was dissolved in water (100 mL) and extracted with $CHCl_3$ (3 × 100 mL). The organic layers were pooled, washed with saturated

aqueous $NaHCO_3$ (1 × 100 mL), brine, dried ($MgSO_4$), filtered, and concentrated in vacuo to provide a brown solid. Purification (silica gel, $CHCl_3$) provided **13** (90 mg, 55%) as a yellow solid: IR (film) 3062, 2952, 2839, 1714, 1619, 1566, 1456, 1438, 1256, 1067 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.56 (d, J = 7.56 Hz, 1 H), 8.34 (s, 1 H), 8.23 (d, J = 8.64 Hz, 1 H), 7.91 (d, J = 8.19 Hz, 1 H), 7.90-7.71 (m, 3 H), 7.68 (s, 1 H), 7.66-7.56 (m, 2 H), 5.38 (d, J = 1.07 Hz, 2 H); ESIMS *m/z* (rel intensity) 285.2 (100, MH^+). Anal. Calcd for $C_{19}H_{12}N_2O \cdot 0.25H_2O$: C, 79.01; H, 5.46; N, 9.70. Found: C, 79.30; H, 5.36; N, 9.66.

Example 6. 11-(4'-Chlorobutylidene)-5,6-dihydro-2,3-dimethoxy-6-methyl-8,9-methylenedioxy-5-oxo-11*H*-indeno[1,2-*c*]isoquinoline (18).

$TiCl_4 \cdot 2THF$ (508 mg, 1.52 mmol), Zn dust (199 mg, 3.04 mmol), and dry THF (15 mL) were added to a flame-dried two-necked flask equipped with a magnetic stir bar and reflux condenser. The suspension was heated at reflux under argon for 3 h, after which a solution of 4-chlorobutanal (**14**) (Li et al., "Synthesis of the Tricyclic ABC Ring Subunit of Mazamine A," *Tetrahedron*, *54*, 6661-6676 (1998), the disclosure of which is incorporated herein by reference) (108.1 mg, 1.01 mmol) and indenoisoquinoline **2** (185 mg, 0.51 mmol) in dry THF (15 mL) was introduced by syringe. The reaction mixture was heated at reflux for 2.5 h, after which 4 N HCl (20 mL) was added. The solution was stirred at room temperature for 1 h and then allowed to stand for 3 h. The resulting orange precipitate was collected by vacuum filtration. The solid was purified by flash chromatography (silica gel, 5:1 $CHCl_3$/hexanes) to provide **18** (96.1 mg, 43%) as an orange solid: mp 196-201 °C; IR (film) 2926, 1636, 1610, 1517, 1483, 1255, 1034 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.89 (s, 1 H), 7.78 (s, 1 H), 7.40 (s, 1 H), 7.37 (s, 1 H), 6.88 (t, J = 7.19 Hz, 1 H), 6.04 (s, 2 H), 4.05 (s, 3 H), 4.03 (s, 3 H), 4.02 (s, 3 H), 3.74 (t, J = 6.32 Hz, 2 H), 3.00 (dt, J = 7.30 and 7.45 Hz, 2 H), 2.20 (qn, J = 6.90 Hz, 2 H); ESIMS *m/z* (rel intensity) 440.7 (100, MH^+), 442.6 (43, MH^+). Anal. Calcd for $C_{24}H_{22}ClNO_5$: C, 65.53; H, 5.04; N, 3.18. Found: C, 65.30; H, 4.96; N, 3.08.

Example 7. 11-(4'-Bromobutylidene)-5,6-dihydro-2,3-dimethoxy-6-methyl-8,9-methylenedioxy-5-oxo-11*H*-indeno[1,2-*c*]isoquinoline (19).

A 100 mL two-necked round-bottomed flask equipped with a magnetic stirring bar, reflux condenser, septa, and argon line was charged with zinc dust (537

mg, 8.21 mmol) and was flame dried. THF (30 mL) and a 1 M solution of $TiCl_4$ in toluene (4.11 mL, 4.11 mmol) were added. The suspension was heated at reflux for 5 h, at which point a suspension of **2** (500 mg, 1.37 mmol) and 4-bromobutanal (**15**) (Canan Koch & Chamberlin, "Enantioselective Preparation of β-Alkyl-γ-butyrolactones from Functionalized Ketene Dithioacetals," *J. Org. Chem.*, *58*, 2725-2737 (1993); Somekawa et al., "Intramolecular [2 + 2]Photocycloadditions of 1-(ω-Alkenyl)-2-pyridones Possessing an Ester Group on the Olefinic Carbon Chain," *J. Org. Chem*, *57*, 5708-5712, (1992), the disclosures of which are incorporated herein by reference) (413 mg, 2.74 mmol) in THF (30 mL) was added by pipette. The reaction mixture was heated at reflux for 1 h and then quenched with 4 N HCl (40 mL). The solution was stirred for 1 h and then cooled at 0 °C for 2 h. The orange precipitate was collected by vacuum filtration to provide an orange solid. This was purified by flash chromatography (silica gel, $CHCl_3$) to provide **19** (161.6 mg, 30%) as an orange solid: mp 197-199 °C; IR (film) 2921, 1610, 1517, 1483, 1381, 1296, 1253, 1207, 1033 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.88 (s, 1 H), 7.44 (s, 1 H), 7.40 (s, 1 H), 7.36 (s, 1 H), 6.83 (t, J = 7.11 Hz, 1 H), 6.05 (s, 2 H), 4.00 (s, 6 H), 3.97 (s, 3 H), 3.57 (t, J = 6.33 Hz, 2 H), 2.97 (q, J = 7.23 Hz, 2 H), 2.25 (qn, J = 6.80 Hz, 2 H); ESIMS *m/z* (rel intensity) 486.2 (97, MH^+), 484.2 (100, MH^+). Anal. Calcd for $C_{24}H_{22}BrNO_5$: C, 59.52; H, 4.58; N, 2.89. Found: C, 59.56; H, 4.56; N, 2.88.

Example 8. 11-(5'-Bromopentylidene)-5,6-dihydro-2,3-dimethoxy-6-methyl-8,9-methylenedioxy-5-oxo-11*H*-indeno[1,2-*c*]isoquinoline (**20**).

A 100 mL two-necked round-bottomed flask equipped with a reflux condenser and magnetic stir bar was charged with zinc dust (537 mg, 8.21 mmol) and flame dried. THF (30 mL) and 1 M $TiCl_4$ in toluene (4.11 mL, 4.11 mmol) were added to the round-bottomed flask and the mixture was heated at reflux for 6 h. THF (30 mL), 5-bromopentanal (**16**) (452 mg, 2.74 mmol) and **2** (500 mg 1.37 mmol) were added to the reaction mixture, which was heated at reflux for 2 h. The reaction mixture was cooled to room temperature, 4 N HCl (40 mL) was added, and this mixture was stirred for 30 min and cooled in a –20 °C freezer overnight. The resulting yellow precipitate was collected by vacuum filtration and purified by flash chromatography to provide **20** (133.7 mg, 33%) as an orange solid: mp 196-197 °C; IR (film) 2932, 1632, 1612, 1517, 1483, 1254, 1032 cm^{-1}; ^1H NMR (500 MHz,

$CDCl_3$) δ 7.89 (s, 1 H), 7.46 (s, 1 H), 7.41 (s, 1 H), 7.32 (s, 1 H), 6.86 (t, J = 6.83 Hz, 1 H), 6.05 (s, 2 H), 4.05 (s, 3 H), 4.03 (s, 3 H), 4.01 (s, 3 H), 3.49 (t, J = 6.47 Hz, 2 H), 2.86 (q, J = 7.15 Hz, 2 H), 2.06 (m, 2 H), 1.88 (m, 2 H); ESIMS *m/z* (rel intensity) 500.2 (95, MH^+), 498.2 (100, MH^+). Anal. Calcd for $C_{25}H_{24}BrNO_5$: C, 60.25; H, 4.85; N, 2.81. Found: C, 60.57; H, 4.90; N, 2.83.

Example 9. 11-(6'-Bromohexylidene)-5,6-dihydro-2,3-dimethoxy-6-methyl-8,9-methylenedioxy-5-oxo-11*H*-indeno[1,2-*c*]isoquinoline (21).

A 100 mL two-necked round-bottomed flask equipped with a reflux condenser and magnetic stir bar was charged with zinc dust (537 mg, 8.21 mmol) and flame dried. THF (30 mL) and 1 M $TiCl_4$ in toluene (4.11 mL, 4.11 mmol) were added to the round-bottomed flask and the mixture was heated at reflux for 4 h. Anhydrous THF (30 mL), 6-bromohexanal (17) (491 mg, 2.74 mmol) and 2 (500 mg 1.37 mmol) were added to the reaction mixture, which was heated at reflux for 2 h. The reaction mixture was cooled to room temperature, 4 N HCl (40 mL) was added, and this mixture was stirred for 30 min and then cooled in a –20 °C freezer overnight. The resulting yellow precipitate was collected by vacuum filtration and purified by flash chromatography to provide 21 (118.0 mg, 17%) as an orange solid: mp 182-185 °C; IR (film) 2929, 1636, 1610, 1516, 1482, 1296, 1255, 1033 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.88 (s, 1 H), 7.46 (s, 1 H), 7.40 (s, 1 H), 7.32 (s, 1 H), 6.88 (t, J = 6.96 Hz, 1 H), 6.05 (s, 2 H), 4.06 (s, 3 H), 4.02 (s, 3 H), 4.00 (s, 3 H), 3.44 (t, J = 6.62 Hz, 2 H), 2.84 (q, J = 7.13 Hz, 2 H), 1.95 (qn, J = 7.04 Hz, 2 H), 1.74 (m, 2 H) 1.66 (m, 2 H); ESIMS *m/z* (rel intensity) 514.2 (100, MH^+), 512.2 (91, MH^+). Anal. Calcd for $C_{26}H_{26}BrNO_5$: C, 60.95; H, 5.11; N, 2.73. Found: C, 60.55; H, 5.08; N, 2.72.

Example 10. 11-(4'-Iodobutylidene)-5,6-dihydro-2,3-dimethoxy-6-methyl-8,9-methylenedioxy-5-oxo-11*H*-indeno[1,2-*c*]isoquinoline (24).

NaI (217 mg, 1.45 mmol) was added to a suspension of bromide 19 (70 mg, 0.15 mmol) in acetone (15 mL). The reaction mixture was heated at reflux for 12 h, after which the resulting orange precipitate was collected by vacuum filtration and purified by flash chromatography (silica gel, $CHCl_3$) to provide 24 (73.0 mg, 95%) as an orange solid: mp 173-174.5 °C; IR (KBr) 2944, 1612, 1522, 1481, 1254, 1033 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.88 (s, 1 H), 7.43 (s, 1 H), 7.40 (s, 1 H), 7.36 (s, 1 H), 6.84 (t, J = 7.22 Hz, 1 H), 6.05 (s, 2 H), 4.08 (s, 3 H), 4.04 (s, 3 H), 4.00 (s, 3 H),

3.35 (t, J = 6.69 Hz, 2 H), 2.95 (q, J = 7.32 Hz, 2 H), 2.19 (m, 2 H); ESIMS *m/z* (rel intensity) 532.1 (100, MH^+). Anal. Calcd for $C_{24}H_{22}INO_5$: C, 54.25; H, 4.17; N, 2.64. Found: C, 54.64; H, 4.25; N, 2.60.

Example 11. 11-(3'-Aminopropylidene)-5,6-dihydro-2,3-dimethoxy-6-methyl-8,9-methylenedioxy-5-oxo-11*H*-indeno[1,2-*c*]isoquinoline (26).

$TiCl_4$-THF (1:2) complex (730 mg, 2.19 mmol) and zinc dust (284 mg, 4.37 mmol) were put in a three-necked round-bottomed flask. THF (30 mL) was added. The resulting suspension was heated under reflux for 4 h. At this point, a mixture of aldehyde **25** (Blaney et al., "Fused and Bridged Bi- and Tri-Cyclic Lactams via Sequential Metallo-Azomethine Ylide Cycloaddition-Lactamisation," *Tetrahedron*, *58*, 1719-37 (2002), the disclosures of which are incorporated herein by reference) (189 mg, 1.09 mmol) and indenoisoquinoline **2** (266 mg, 0.73 mmol) in THF (30 mL) was added via syringe. The reaction mixture was stirred under reflux for an additional 4 h. Then 3 N HCl (10 mL) was added after cooling to room temperature and the mixture was stirred at room temperature for 1 h, followed by 0 °C for 2 h, and finally at room temperature overnight. The mixture was cooled to 0 °C and solid $NaHCO_3$ was added to neutralize HCl. The solvents were evaporated and the residue was subjected to flash chromatography, eluting with $CHCl_3$-MeOH (4:1) to provide **26** as a yellow powder (121 mg, 41%): mp >180 °C (dec); ^{1}H NMR (300 MHz, DMSO-d_6) 7.68 (s, 1 H), 7.62 (s, 1 H), 7.51 (s, 1 H), 7.48 (s, 1 H), 6.94 (t, J = 6.0 Hz, 1 H), 6.15 (s, 2 H), 4.01 (s, 3 H), 3.95 (s, 3 H), 3.87 (s, 3 H), 3.12-3.18 (m, 4 H). ESIMS *m/z* (rel intensity) 407.0 (100, MH^+). Anal. Calcd for $C_{23}H_{22}N_2O_5 \cdot 0.9CHCl_3$: C, 55.86; H, 4.49; N, 5.45. Found: C, 55.86; H, 4.72; N, 5.24.

Example 12. Top1-Mediated DNA Cleavage Reactions.

Human recombinant top1 was purified from Baculovirus as described in Pourquier et al., "Induction of Reversible Complexes between Eukaryotic DNA Topoisomerase I and DNA-containing Oxidative Base Damages. 7,8-Dihydro-8-Oxoguanine and 5-Hydroxycytosine," *J. Biol. Chem.*, *274*, 8516-23 (1999), the disclosure of which is incorporated herein by reference. The 161 bp fragment from pBluescript SK(-) phagemid DNA (Stratagene, La Jolla, CA) was cleaved with the restriction endonuclease Pvu II and Hind III (New England Biolabs, Beverly, MA) in

supplied NE buffer 2 (10 μL reactions) for 1 h at 37 °C, and separated by electrophoresis in a 1% agarose gel made in 1X TBE buffer. The 161 bp fragment was eluted from the gel slice (centrilutor by Amicon) and concentrated in a centricon 50 centrifugal concentrator (Amicon, Beverly, MA). Approximately 200 ng of the fragment was 3'-end labeled at the Hind III site by fill-in reaction with [alpha-^{32}P]-dGTP and 0.5 mM dATP, dCTP, and dTTP, in React 2 buffer (50 mM Tris-HCl, pH 8.0, 100 mM $MgCl_2$, 50 mM NaCl) with 0.5 units of DNA polymerase I (Klenow fragment). Unincorporated ^{32}P-dGTP was removed using mini Quick Spin DNA columns (Roche, Indianapolis, IN), and the eluate containing the 3'-end-labeled 161 bp fragment was collected. Aliquots (approximately 50,000 dpm/reaction) were incubated with top1 at 22 °C for 30 min in the presence of the tested drug. Reactions were terminated by adding SDS (0.5% final concentration). The samples (10 μL) were mixed with 30 μL of loading buffer (80% formamide, 10 mM sodium hydroxide, 1 mM sodium EDTA, 0.1% xylene cyanol, and 0.1% bromophenol blue, pH 8.0). Aliquots were separated in denaturing gels (16% polyacrylamide, 7 M urea). Gels were dried and visualized by using a Phosphoimager and ImageQuant software (Molecular Dynamics, Sunnyvale, CA).

The forgoing examples are intended to illustrate exemplary embodiments of the invention described herein and are not intended to limit the scope of the invention. Additional understanding of the nature of the invention may be discerned from examination of the attached Exhibit A, the description of which is incorporated herein by reference.

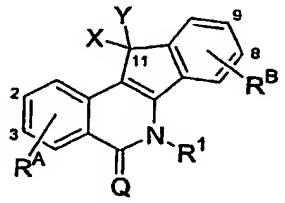
Table 1. Cytotoxicities and Topoisomerase I Inhibitory Activities of Indenoisoquinoline Analogs.

	cytotoxicity (GI50 in μM)[a]									
compd	lung HOP-62	colon HCT-116	CNS SF-539	melanoma UACC-62	ovarian OVCAR-3	renal SN12C	prostate DU-145	breast MDA-MB-435	MGM[b]	Top 1 Cleavage[c]
8	>100	57.3	>100	>100	>100	>100	>100	>100	91.2	+
13	68.2	32.7	66.7	97.2	39.8	>100	>100	41.8	58.9	++
18	17.5	40.4	NT[d]	33.3	42.3	>100	35.9	>100	33.1	+
19	19.4	37.8	30.0	11.4	58.1	>100	67.8	>100	27.8	0
20	26.6	9.5	7.1	>100	>100	>100	4.5	>100	33.1	±
21	3.1	6.1	5.7	3.9	23.6	5.7	5.5	19.2	7.8	+
24	27.6	0.56	4.3	3.5	22.8	8.8	28.7	5.2	16.8	0
26	0.071	0.028	0.42	0.20	0.56	0.58	0.37	1.8	0.34	+++
2	1.3	35	41	4.2	73	68	37	96	20	++
7	0.06	0.13	0.26	0.25	0.31	0.31	0.04	1.21	0.16	+++

[a] The cytotoxicity GI50 values are the concentrations corresponding to 50% growth inhibition. [b] Mean graph midpoint for growth inhibition of all human cancer cell lines successfully tested. [c]The compounds were tested at concentrations ranging up to 10 μM. The activity of the compounds to produce top1-mediated DNA cleavage was expressed semi-quantitatively as follows: +: weak activity; ++: similar activity as the parent compound **2**; +++ & ++++: greater activity than the parent compound **2**; ++++: similar activity as camptothecin (**3**).

WHAT IS CLAIMED IS:

1. A compound having the formula:



where Q is oxygen or sulfur;

X is hydrogen and Y is CR^2R^3, or X and Y are taken together to form $=CR^2R^3$; $=NR^2$; $=NOR^2$; or $=NNR^2R^3$;

R^1, R^2, and R^3 are each independently selected from the group consisting of hydrogen and a radical $-(CH_2)_mZ$, where m is 0-6 and Z is selected from the group consisting of halogen, hydroxy, formyl, C_1-C_6 alkanoyloxy, optionally-substituted benzoyloxy, C_1-C_6 alkyl, C_1-C_6 alkoxy, C_3-C_8 cycloalkyl, C_3-C_8 cycloalkoxy, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_1-C_6 haloalkoxy, C_3-C_8 halocycloalkyl, C_3-C_8 halocycloalkoxy, amino, C_1-C_6 alkylamino, (C_1-C_6 alkyl)(C_1-C_6 alkyl)amino, amido, N-(C_1-C_6 alkyl)amido, cyano, nitro, C_1-C_6 alkylsulfonyl, optionally-substituted phenyl, and optionally-substituted phenoxy; or Z is selected from the group consisting of $-CO_2R^4$ and $-CONR^5R^6$, where R^4, R^5, and R^6 are each independently selected from the group consisting of hydrogen, C_1-C_6 alkyl, C_3-C_8 cycloalkyl, C_1-C_6 haloalkyl, optionally-substituted phenyl, and optionally-substituted phenyl-C_1-C_6 alkyl; or

when X is NNR^2R^3, R^2 and R^3 are taken together with the attached nitrogen to form an optionally-substituted heterocycle;

R^A represents 1-4 substituents each independently selected from the group consisting of hydrogen and a radical $-(CH_2)_{m'}Z'$, where m' is 0-6 and Z' is selected from the group consisting of halogen, hydroxy, C_1-C_6 alkanoyloxy, optionally-substituted benzoyloxy, C_1-C_6 alkyl, C_1-C_6 alkoxy, C_3-C_8 cycloalkyl, C_3-C_8 cycloalkoxy, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_1-C_6 haloalkoxy, C_3-C_8 halocycloalkyl, C_3-C_8 halocycloalkoxy, amino, C_1-C_6 alkylamino, (C_1-C_6 alkyl)(C_1-C_6 alkyl)amino, amido, N-(C_1-C_6 alkyl)amido, cyano, nitro, C_1-C_6 alkylsulfonyl, optionally-substituted phenyl, and optionally-substituted phenoxy; or Z' is selected from the group consisting of $-CO_2R^{4'}$ and $-CONR^{5'}R^{6'}$, where $R^{4'}$, $R^{5'}$, and $R^{6'}$ are each

independently selected from the group consisting of hydrogen, C_1-C_6 alkyl, C_3-C_8 cycloalkyl, C_1-C_6 haloalkyl, optionally-substituted phenyl, and optionally-substituted phenyl-C_1-C_6 alkyl; or

R^A represents 2-4 substituents where 2 of said substituents are adjacent substituents and are taken together with the attached carbons to form an optionally-substituted carbocycle or an optionally-substituted heterocycle, and the remaining 2 substituents are each independently selected from the group consisting of hydrogen and a radical $-(CH_2)_{m'}Z'$, where m' is 0-6 and Z' is selected from the group consisting of hydrogen, halogen, hydroxy, C_1-C_6 alkanoyloxy, optionally-substituted benzoyloxy, C_1-C_6 alkyl, C_1-C_6 alkoxy, C_3-C_8 cycloalkyl, C_3-C_8 cycloalkoxy, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_1-C_6 haloalkoxy, C_3-C_8 halocycloalkyl, C_3-C_8 halocycloalkoxy, amino, C_1-C_6 alkylamino, (C_1-C_6 alkyl)(C_1-C_6 alkyl)amino, amido, N-(C_1-C_6 alkyl)amido, cyano, nitro, C_1-C_6 alkylsulfonyl, optionally-substituted phenyl, and optionally-substituted phenoxy; or Z' is selected from the group consisting of $-CO_2R^{4'}$ and $-CONR^{5'}R^{6'}$, where $R^{4'}$, $R^{5'}$, and $R^{6'}$ are each independently selected from the group consisting of hydrogen, C_1-C_6 alkyl, C_3-C_8 cycloalkyl, C_1-C_6 haloalkyl, optionally-substituted phenyl, and optionally-substituted phenyl-C_1-C_6 alkyl; and

R^B represents 1-4 substituents each independently selected from the group consisting of hydrogen and a radical $-(CH_2)_{m''}Z''$, where m'' is 0-6 and Z'' is selected from the group consisting of halogen, hydroxy, C_1-C_6 alkanoyloxy, optionally-substituted benzoyloxy, C_1-C_6 alkyl, C_1-C_6 alkoxy, C_3-C_8 cycloalkyl, C_3-C_8 cycloalkoxy, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_1-C_6 haloalkoxy, C_3-C_8 halocycloalkyl, C_3-C_8 halocycloalkoxy, amino, C_1-C_6 alkylamino, (C_1-C_6 alkyl)(C_1-C_6 alkyl)amino, amido, N-(C_1-C_6 alkyl)amido, cyano, nitro, C_1-C_6 alkylsulfonyl, optionally-substituted phenyl, and optionally-substituted phenoxy; or Z'' is selected from the group consisting of $-CO_2R^{4''}$ and $-CONR^{5''}R^{6''}$, where $R^{4''}$, $R^{5''}$, and $R^{6''}$ are each independently selected from the group consisting of hydrogen, C_1-C_6 alkyl, C_3-C_8 cycloalkyl, C_1-C_6 haloalkyl, optionally-substituted phenyl, and optionally-substituted phenyl-C_1-C_6 alkyl; or

R^B represents 2-4 substituents where 2 of said substituents are adjacent substituents and are taken together with the attached carbons to form an optionally-

substituted carbocycle or an optionally-substituted heterocycle, and the remaining 2 substituents are each independently selected from the group consisting of hydrogen and a radical $-(CH_2)_{m''}Z''$, where m'' is 0-6 and Z'' is selected from the group consisting of hydrogen, halogen, hydroxy, C_1-C_6 alkanoyloxy, optionally-substituted benzoyloxy, C_1-C_6 alkyl, C_1-C_6 alkoxy, C_3-C_8 cycloalkyl, C_3-C_8 cycloalkoxy, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_1-C_6 haloalkoxy, C_3-C_8 halocycloalkyl, C_3-C_8 halocycloalkoxy, amino, C_1-C_6 alkylamino, (C_1-C_6 alkyl)(C_1-C_6 alkyl)amino, amido, N-(C_1-C_6 alkyl)amido, cyano, nitro, C_1-C_6 alkylsulfonyl, optionally-substituted phenyl, and optionally-substituted phenoxy; or Z'' is selected from the group consisting of $-CO_2R^{4''}$ and $-CONR^{5''}R^{6''}$, where $R^{4''}$, $R^{5''}$, and $R^{6''}$ are each independently selected from the group consisting of hydrogen, C_1-C_6 alkyl, C_3-C_8 cycloalkyl, C_1-C_6 haloalkyl, optionally-substituted phenyl, and optionally-substituted phenyl-C_1-C_6 alkyl.

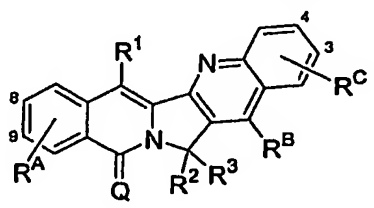
2. The compound of claim 1, wherein Q is oxygen, R^A is 2,3-bis(C_1-C_6 alkoxy), R^B is 8,9-alkylenedioxy, and X and Y are taken together to form $=CR^2R^3$, where R^2 is hydrogen.

3. The compound of claim 1, wherein Q is oxygen, R^A is 2,3-bis(C_1-C_6 alkoxy), R^B is 8,9-alkylenedioxy, X and Y are taken together to form $=CR^2R^3$, R^2 is hydrogen, and R^1 is hydrogen, C_1-C_6 alkyl, C_3-C_8 cycloalkyl, C_1-C_6 haloalkyl, C_3-C_8 halocycloalkyl, amino-C_1-C_6 alkyl, C_1-C_6 alkylamino-C_1-C_6 alkyl, or (C_1-C_6 alkyl)(C_1-C_6 alkyl)amino-C_1-C_6 alkyl.

4. A pharmaceutical composition comprising a compound of claim 1 and a pharmaceutically acceptable carrier, excipient, or diluent therefor.

5. A method for treating a mammal in need of relief from a disease state including cancer, comprising administering to the mammal an effective amount of a compound according to claim 1 or of a pharmaceutical composition according to claim 4.

6. A compound having the formula:



where Q is oxygen or sulfur;

R^1, R^2, and R^3 are each independently selected from the group consisting of hydrogen and a radical $-(CH_2)_mZ$, where m is 0-6 and Z is selected from the group consisting of halogen, hydroxy, formyl, C_1-C_6 alkanoyloxy, optionally-substituted benzoyloxy, C_1-C_6 alkyl, C_1-C_6 alkoxy, C_3-C_8 cycloalkyl, C_3-C_8 cycloalkoxy, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_1-C_6 haloalkoxy, C_3-C_8 halocycloalkyl, C_3-C_8 halocycloalkoxy, amino, C_1-C_6 alkylamino, (C_1-C_6 alkyl)(C_1-C_6 alkyl)amino, amido, N-(C_1-C_6 alkyl)amido, cyano, nitro, C_1-C_6 alkylsulfonyl, optionally-substituted phenyl, and optionally-substituted phenoxy; or Z is selected from the group consisting of $-CO_2R^4$ and $-CONR^5R^6$, where R^4, R^5, and R^6 are each independently selected from the group consisting of hydrogen, C_1-C_6 alkyl, C_3-C_8 cycloalkyl, C_1-C_6 haloalkyl, optionally-substituted phenyl, and optionally-substituted phenyl-C_1-C_6 alkyl; or

R^1 is selected from the group consisting of hydrogen and a radical $-(CH_2)_mZ$, where m is 0-6 and Z is selected from the group consisting of halogen, hydroxy, formyl, C_1-C_6 alkanoyloxy, optionally-substituted benzoyloxy, C_1-C_6 alkyl, C_1-C_6 alkoxy, C_3-C_8 cycloalkyl, C_3-C_8 cycloalkoxy, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_1-C_6 haloalkoxy, C_3-C_8 halocycloalkyl, C_3-C_8 halocycloalkoxy, amino, C_1-C_6 alkylamino, (C_1-C_6 alkyl)(C_1-C_6 alkyl)amino, amido, N-(C_1-C_6 alkyl)amido, cyano, nitro, C_1-C_6 alkylsulfonyl, optionally-substituted phenyl, and optionally-substituted phenoxy; or Z is selected from the group consisting of $-CO_2R^4$ and $-CONR^5R^6$, where R^4, R^5, and R^6 are each independently selected from the group consisting of hydrogen, C_1-C_6 alkyl, C_3-C_8 cycloalkyl, C_1-C_6 haloalkyl, optionally-substituted phenyl, and optionally-substituted phenyl-C_1-C_6 alkyl; and R^2 and R^3 are taken together with the attached carbon to form an optionally-substituted carbocycle or heterocycle;

R^A represents 1-4 substituents each independently selected from the group consisting of hydrogen and a radical $-(CH_2)_{m'}Z'$, where m' is 0-6 and Z' is selected from the group consisting of halogen, hydroxy, C_1-C_6 alkanoyloxy, optionally-substituted benzoyloxy, C_1-C_6 alkyl, C_1-C_6 alkoxy, C_3-C_8 cycloalkyl, C_3-C_8 cycloalkoxy, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_1-C_6 haloalkoxy, C_3-C_8 halocycloalkyl, C_3-C_8 halocycloalkoxy, amino, C_1-C_6 alkylamino, (C_1-C_6 alkyl)(C_1-C_6

alkyl)amino, amido, N-(C_1-C_6 alkyl)amido, cyano, nitro, C_1-C_6 alkylsulfonyl, optionally-substituted phenyl, and optionally-substituted phenoxy; or Z′ is selected from the group consisting of -$CO_2R^{4'}$ and -$CONR^{5'}R^{6'}$, where $R^{4'}$, $R^{5'}$, and $R^{6'}$ are each independently selected from the group consisting of hydrogen, C_1-C_6 alkyl, C_3-C_8 cycloalkyl, C_1-C_6 haloalkyl, optionally-substituted phenyl, and optionally-substituted phenyl-C_1-C_6 alkyl; or

R^A represents 2-4 substituents where 2 of said substituents are adjacent substituents and are taken together with the attached carbons to form an optionally-substituted carbocycle or an optionally-substituted heterocycle, and the remaining 2 substituents are each independently selected from the group consisting of hydrogen and a radical -$(CH_2)_{m'}Z'$, where m′ is 0-6 and Z′ is selected from the group consisting of hydrogen, halogen, hydroxy, C_1-C_6 alkanoyloxy, optionally-substituted benzoyloxy, C_1-C_6 alkyl, C_1-C_6 alkoxy, C_3-C_8 cycloalkyl, C_3-C_8 cycloalkoxy, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_1-C_6 haloalkoxy, C_3-C_8 halocycloalkyl, C_3-C_8 halocycloalkoxy, amino, C_1-C_6 alkylamino, (C_1-C_6 alkyl)(C_1-C_6 alkyl)amino, amido, N-(C_1-C_6 alkyl)amido, cyano, nitro, C_1-C_6 alkylsulfonyl, optionally-substituted phenyl, and optionally-substituted phenoxy; or Z′ is selected from the group consisting of -$CO_2R^{4'}$ and -$CONR^{5'}R^{6'}$, where $R^{4'}$, $R^{5'}$, and $R^{6'}$ are each independently selected from the group consisting of hydrogen, C_1-C_6 alkyl, C_3-C_8 cycloalkyl, C_1-C_6 haloalkyl, optionally-substituted phenyl, and optionally-substituted phenyl-C_1-C_6 alkyl;

R^B is selected from the group consisting of hydrogen and a radical -$(CH_2)_{m''}Z''$, where m″ is 0-6 and Z″ is selected from the group consisting of halogen, hydroxy, C_1-C_6 alkanoyloxy, optionally-substituted benzoyloxy, C_1-C_6 alkyl, C_1-C_6 alkoxy, C_3-C_8 cycloalkyl, C_3-C_8 cycloalkoxy, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_1-C_6 haloalkoxy, C_3-C_8 halocycloalkyl, C_3-C_8 halocycloalkoxy, amino, C_1-C_6 alkylamino, (C_1-C_6 alkyl)(C_1-C_6 alkyl)amino, amido, N-(C_1-C_6 alkyl)amido, cyano, nitro, C_1-C_6 alkylsulfonyl, optionally-substituted phenyl, and optionally-substituted phenoxy; or Z″ is selected from the group consisting of -$CO_2R^{4''}$ and -$CONR^{5''}R^{6''}$, where $R^{4''}$, $R^{5''}$, and $R^{6''}$ are each independently selected from the group consisting of hydrogen, C_1-C_6 alkyl, C_3-C_8 cycloalkyl, C_1-C_6 haloalkyl, optionally-substituted phenyl, and optionally-substituted phenyl-C_1-C_6 alkyl; and′

R^C represents 1-4 substituents each independently selected from the group consisting of hydrogen and a radical $-(CH_2)_{m'''}Z'''$, where m''' is 0-6 and Z''' is selected from the group consisting of halogen, hydroxy, C_1-C_6 alkanoyloxy, optionally-substituted benzoyloxy, C_1-C_6 alkyl, C_1-C_6 alkoxy, C_3-C_8 cycloalkyl, C_3-C_8 cycloalkoxy, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_1-C_6 haloalkoxy, C_3-C_8 halocycloalkyl, C_3-C_8 halocycloalkoxy, amino, C_1-C_6 alkylamino, (C_1-C_6 alkyl)(C_1-C_6 alkyl)amino, amido, N-(C_1-C_6 alkyl)amido, cyano, nitro, C_1-C_6 alkylsulfonyl, optionally-substituted phenyl, and optionally-substituted phenoxy; or Z'' is selected from the group consisting of $-CO_2R^{4'''}$ and $-CONR^{5'''}R^{6'''}$, where $R^{4'''}$, $R^{5'''}$, and $R^{6'''}$ are each independently selected from the group consisting of hydrogen, C_1-C_6 alkyl, C_3-C_8 cycloalkyl, C_1-C_6 haloalkyl, optionally-substituted phenyl, and optionally-substituted phenyl-C_1-C_6 alkyl; or

R^C represents 2-4 substituents where 2 of said substituents are adjacent substituents and are taken together with the attached carbons to form an optionally-substituted carbocycle or an optionally-substituted heterocycle, and the remaining 2 substituents are each independently selected from the group consisting of hydrogen and a radical $-(CH_2)_{m'''}Z'''$, where m''' is 0-6 and Z''' is selected from the group consisting of hydrogen, halogen, hydroxy, C_1-C_6 alkanoyloxy, optionally-substituted benzoyloxy, C_1-C_6 alkyl, C_1-C_6 alkoxy, C_3-C_8 cycloalkyl, C_3-C_8 cycloalkoxy, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 haloalkyl, C_1-C_6 haloalkoxy, C_3-C_8 halocycloalkyl, C_3-C_8 halocycloalkoxy, amino, C_1-C_6 alkylamino, (C_1-C_6 alkyl)(C_1-C_6 alkyl)amino, amido, N-(C_1-C_6 alkyl)amido, cyano, nitro, C_1-C_6 alkylsulfonyl, optionally-substituted phenyl, and optionally-substituted phenoxy; or Z''' is selected from the group consisting of $-CO_2R^{4'''}$ and $-CONR^{5'''}R^{6'''}$, where $R^{4'''}$, $R^{5'''}$, and $R^{6'''}$ are each independently selected from the group consisting of hydrogen, C_1-C_6 alkyl, C_3-C_8 cycloalkyl, C_1-C_6 haloalkyl, optionally-substituted phenyl, and optionally-substituted phenyl-C_1-C_6 alkyl.

7. The compound of claim 6, wherein Q is oxygen, R^A is 2,3-bis(C_1-C_6 alkoxy), and R^B, R^C, R^1, $R^2$and R^3 are each hydrogen.

8. A pharmaceutical composition comprising a compound of claim 6 and a pharmaceutically acceptable carrier, excipient, or diluent therefor.

9. A method for treating a mammal in need of relief from a disease state including cancer, comprising administering to the mammal an effective amount of a compound according to claim 6 or of a pharmaceutical composition according to claim 8.

5

ABSTRACT

The synthesis and biological activity of benzoisoindoloisoquinoline compounds are described. The synthesis and biological activity of C-11-substituted indenoisoquinolines is also described. Indenoisoquinolines substituted at C-11 are prepared by McMurry reactions of 11-ketoindenoisoquinolines with aldehydes.

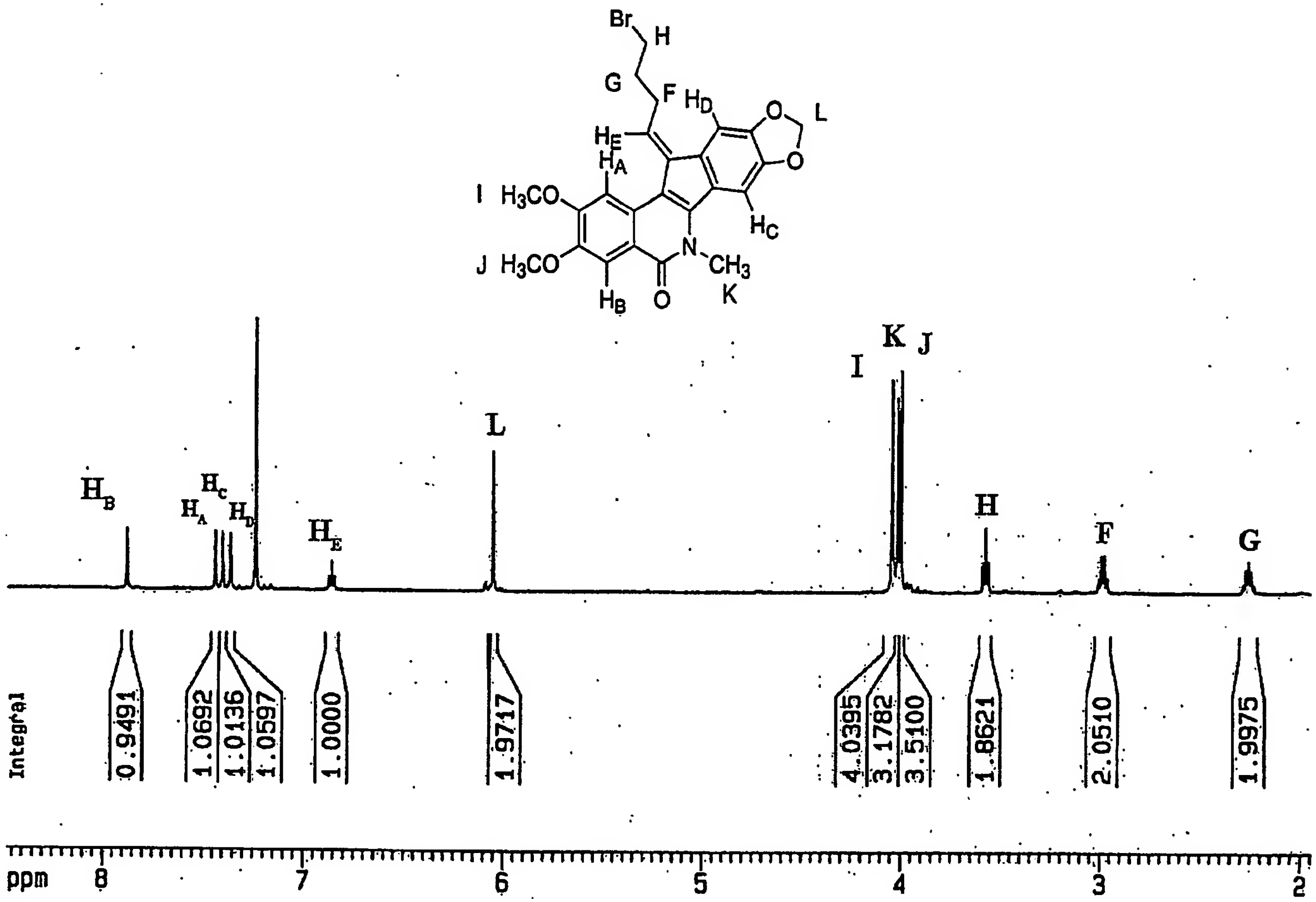


Figure 1. ^{1}H NMR Spectrum of indenoisoquinoline 19.

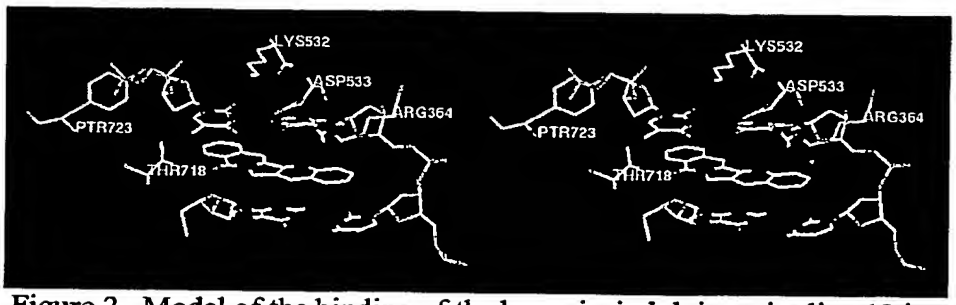


Figure 2. Model of the binding of the benzoisoindoloisoquinoline 13 in the ternary complex consisting of DNA, top1, and the inhibitor. The diagram is programmed for wall-eyed viewing.

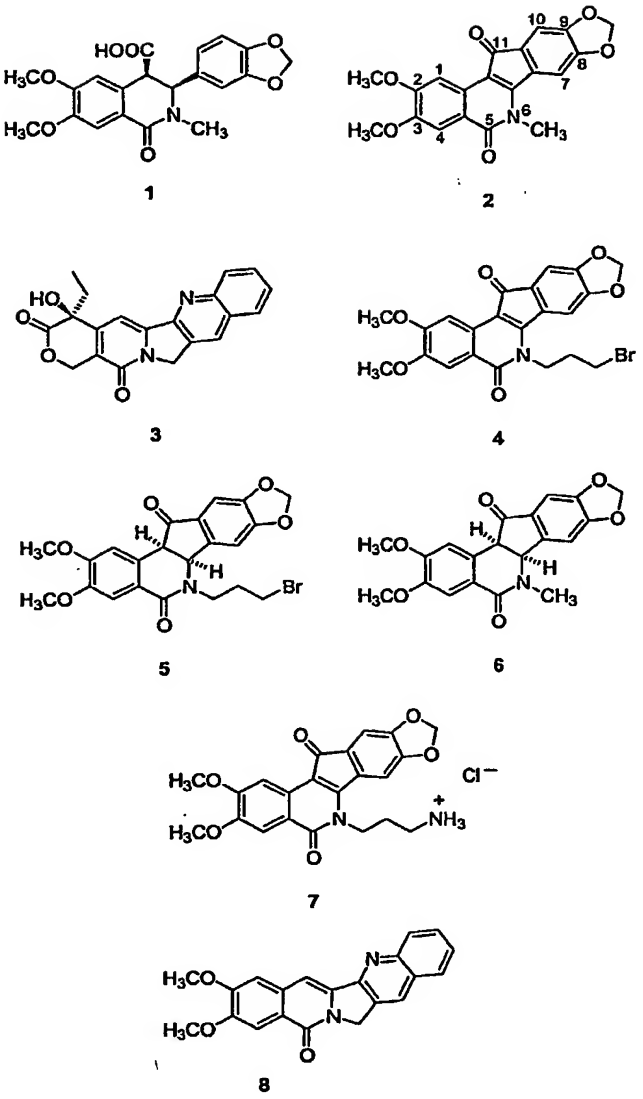


Figure 3.

60469718 .051203

EXHIBIT A

Design, Synthesis, and Biological Evaluation of Cytotoxic 11-Alkenylindenoisoquinoline Topoisomerase I Inhibitors and Indenoisoquinoline-Camptothecin Hybrids

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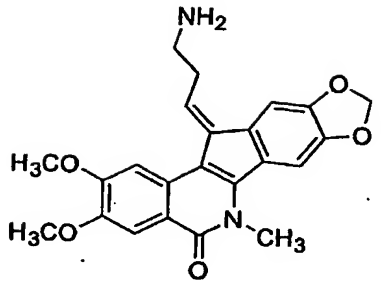
TOC Graphic

[redacted]

Abstract. The indenoisoquinolines are a novel class of topoisomerase I (top1) inhibitors that are cytotoxic in cancer cell cultures and are therefore under development as potential anti-cancer agents. As inhibitors of the DNA religation reaction occurring after DNA cleavage by the enzyme, they are classified as top1 poisons, similar to the camptothecins. Two strategies were employed in order to further develop the structure-activity relationships of the indenoisoquinolines and enhance their therapeutic potential. The first strategy involved the synthesis of indenoisoquinoline-camptothecin hybrid molecules to take advantage of a proposed structural analogy between the indenoisoquinolines and camptothecin. The desired hybrids were synthesized by reaction of halogenated phthalides with a dihydropyrroloquinoline. The second strategy involved the attachment of various alkenyl substituents to the C-11 position of the indenoisoquinolines, which were assumed to project into the DNA minor groove. The required C-11-substituted indenoisoquinolines were synthesized by McMurry reactions of 11-ketoindenoisoquinolines with aldehydes, and the geometry of the resulting alkenes was established by nuclear Overhauser effect (NOE) difference NMR spectroscopy. All of the new indenoisoquinolines were examined for cytotoxicity in human cancer cell cultures as well as for activity vs. top1. Although the indenoisoquinoline-camptothecin hybrid molecules proved to be less cytotoxic and displayed less activity against top1, an analogue incorporating a 3'-aminoalkenyl substituent at the C-11 position of the indenoisoquinoline system was significantly more potent than the prototype indenoisoquinoline in both assays. These results indicate that C-11 aminoalkyl substituents that are assumed to project into the minor groove enhance the cytotoxicity and top1 inhibitory activity of the parent indenoisoquinoline system.


NH2
O
O
H3CO
H3CO
N
CH3
O

Introduction

As previously described in 1978, treatment of the cis-substituted isoquinolone **1** with thionyl chloride unexpectedly afforded the indenoisoquinoline **2** instead of the acid chloride of **1**.[1] The product **2** was found to be moderately active as an anticancer agent, but its limited potency did not warrant further investigations. A later COMPARE analysis of the cytotoxicity profile of the topoisomerase I (top1) inhibitor camptothecin (**3**) revealed that it was similar to the cytotoxicity profile produced by the indenoisoquinoline **2** (referred to as NCS314622), suggesting that compound **2** might also be a top1 inhibitor.[2-4] Subsequently, the indenoisoquinoline **2** was indeed found to inhibit top1, and its ability to stabilize the "cleavable complexes" by inhibition of the DNA religation reactions after top1-catalyzed single strand breakage classified it as a top1 "poison" as opposed to a top1 "suppressor".[2] In this respect, the indenoisoquinoline **2** was found to resemble camptothecin (**3**). However, the DNA single-strand breaks induced by the indenoisoquinoline **2** were more stable than those induced by camptothecin (**3**), and the cleavage site specificity of **2** was different from that of camptothecin (**3**).[2]

Although several camptothecin (**3**) derivatives such as irinotecan and topotecan are clinically useful anticancer agents, they suffer from several limitations resulting from instability due to lactone ring opening and rapid reversibility of the cleavage complexes after drug removal. Consequently, there is a present need for additional therapeutic agents that inhibit top1, like the camptothecins, but induce novel DNA cleavage patterns, have modified toxicity profiles and extended durations of action, and display different antitumor spectra relative to the camptothecins themselves. Therefore, a number of analogues of the indenoisoquinoline **2** have been synthesized with the aim of improving their anticancer and enzyme inhibitory potencies.[5-7] Among the modifications that were tried in order to achieve these goals, the replacement of the *N*-methyl group with 3-bromopropyl and 3-aminopropyl substituents produced the desired increase in potency. For example, the 3-bromopropyl analogue **4**[6] was moderately more potent than the parent compound **2**,[5] and the 3-bromopropyl derivative **5**[7] of the cis-fused analogue **6**[5]

was significantly more potent than **6** as a cytotoxic agent and as a top1 inhibitor. Likewise, the hydrochloride **7**[6] of the *N*-3-aminopropyl derivative was significantly more potent as an enzyme inhibitor and as a cytotoxic agent in cancer cell cultures than the parent compound **2**.

In the present investigation, an attempt was made to take advantage of a possible structural relationship between the lead compound **2** and camptothecin (**3**), which is drawn here in an orientation that emphasizes its resemblance to **2**. In this view, the lactam of the lead indenoisoquinoline **2** would correspond to the lactam of camptothecin (**3**), and the two methoxyl oxygens of **2** would correspond to the two lactone oxygens of **3**. We reasoned that if the structural correspondence were indeed responsible for the activity of **2**, then a more potent analogue of **2** might be synthesized by making a compound having a hybrid structure that would more closely resemble camptothecin (**3**). Compound **8** was therefore proposed as a logical hybrid structure based on the calculated electrostatic potential surfaces of **2**, **3**, and **8**, which are displayed in Figure 1.

A second strategy that was pursued involved the attachment of haloalkenyl and aminoalkenyl side chains to C-11, since, as stated above, similar substituents had already proven to be effective in enhancing the anticancer activity and top1 inhibitory activity when attached to the lactam nitrogen of the indenoisoquinolines. Prior DNA unwinding studies had indicated that *N*-3-aminoalkyl derivatives of the indenoisoquinoline ring system can intercalate.[6] If one assumes a similar orientation of the indenoisoquinoline **7** relative to DNA as was observed in the recently published X-ray structure of the topotecan ternary complex,[8] taking into consideration the structural analogy between the two ligands discussed above, the hypothetical model shown in Figure 2 can be constructed. In this proposal, the cationic side chain on the indenoisoquinoline nitrogen would project into the major groove towards the Asn352 residue of the protein. According to this model, the attachment of alkenyl side chains using the C-11 ketone as a reactive functional group would afford indenoisoquinolines having side chains that would project into the minor groove, toward the Arg364 and Asp533 residues of the enzyme. The present study was therefore undertaken in order to determine the biological effects resulting from the

introduction of a variety of substituents at C-11 that would presumably project into the minor groove of DNA.

Chemistry

Syntheses of the indenoisoquinoline-camptothecin analogue **8** and the corresponding compound **13**,[9] which lacks the two methoxyl groups of **8**, are outlined in Scheme 1. Treatment of a THF solution of the dimesylate **9**[10] with liquid ammonia afforded the 2,3-dihydro-1*H*-pyrrolo[3,4-*b*]quinoline (**10**),[11] which was reacted *in situ* with bromide **11**[12] to afford the desired compound **8**. Similarly, reaction of **10** with the chloride **12**[13] yielded the corresponding unsubstituted derivative **13**.[9]

As portrayed in Scheme 2, the 11-indenoisoquinolines **18-21** were prepared using a McMurry reaction of the ketone **2**[1] with the haloaldehydes **14-17**. In each case, the production of a single double bond isomer was observed. The stereochemistry of the double bond was determined by obtaining nuclear Overhauser effect (NOE) difference spectra of compound **19**. To determine the stereochemistry of the double bond of indenoisoquinoline **19** by NOE difference spectra, unambiguous assignments of the signals in the ^{1}H NMR spectrum of **19** (Figure 3) were essential. We were confident in assigning the H_B, H_E, L, F, G, and H protons to the resonances at 7.89, 6.86, 6.08, 3.00, 2.28, and 3.61 ppm, respectively (see Figure 3). The resonance at 7.89 ppm was assigned to H_B because this proton is adjacent to the amide carbonyl, which would deshield H_B and cause it to shift farthest downfield with respect to the other aromatic protons. The resonance at 6.68 ppm was assigned to the vinylic proton H_E because this is the only triplet that should integrate for one proton. Furthermore, the resonance at 6.08 ppm was assigned to the L protons because they are the only protons that should appear as a two-proton singlet. The assignment of protons at F, G, and H was accomplished by evaluating their chemical shifts. The protons at H are adjacent to a bromide and are farthest downfield at 3.60 ppm. The allylic protons at F are further upfield at 3.00 ppm, and finally the protons at G are farthest upfield at 2.28 ppm.

The remaining aromatic resonances were assigned using NOE difference spectrometry. Irradiation of the L protons (6.08 ppm) resulted in enhancement of the resonances at 7.39 and 7.36 ppm, corresponding to H_D and H_C. Because we know that the resonance at 7.89 ppm is for H_B, we can assign the resonance at 7.44 ppm to H_A by the process of elimination. Irradiation of H_E (6.86 ppm) resulted in a strong enhancement of the resonance at 7.44 ppm corresponding to H_A instead of H_D. Therefore, the double bond at C-11 of indenoisoquinoline **4** can be unambiguously assigned the *E* stereochemistry.

The assignments of the remainder of the resonances in the NMR spectrum of **19** (Figure 3) were accomplished in the same manner and supported the assignments made above. Specifically, irradiation of the F protons (3.00 ppm) caused an enhancement of the resonance at 7.36 ppm. Therefore, the resonance at 7.36 ppm corresponds to H_D. Through the process of elimination, the resonance at 7.39 ppm must belong to H_C. Irradiation of H_C (7.39 ppm) resulted in enhancement of the resonance at 4.040 ppm, resulting in assignment of this resonance to the protons of the methyl amine K. Finally, irradiation of H_B (7.89 ppm) resulted in enhancement of the resonance at 4.023 ppm. Therefore, the resonance at 4.023 ppm corresponds to the protons at J, and by process of elimination, the resonance at 4.044 belongs to the protons at I.

The fact that only one double bond isomer having the *E* geometry is produced in each case seems reasonable because inspection of molecular models suggests that the allylic carbon is closer to C-1 in the *Z* isomer than it is to C-10 in the *E*-isomer (see structure **23** for numbering scheme). The steric clash between the hydrogens attached to the allylic carbon of the *Z* isomers with the hydrogen attached to the C-1 carbon atom should therefore raise its energy relative to the *E* isomers and make the *Z* isomers less accessible during the McMurry coupling reaction. In order to investigate this point further, the global energy minima of the *E* and *Z* isomers **22** and **23** were calculated with the Sybyl® 6.8 program using the Tripos force field and Gasteiger-Hückel charges. The results obtained using the grid search routine indicated a global minimum energy of 32.50 kcal/mol for the *E* isomer **22** and 72.48 kcal/mol for the *Z* isomer **23**. The calculated

energy differences are therefore significant and could explain why none of the *Z* isomer was detected after the McMurry reaction.

The 4-iodobutenyl compound **24** was derived from the bromide **19** using the Finkelstein reaction (Scheme 3). As with the haloalkene derivatives, the McMurry reaction of the lead compound **2** with Boc-protected β-alaninal **(25)**[14] afforded the desired 3-aminopropenyl compound **26** after acidic work-up (Scheme 4).

Biological Results and Discussion

The indenoisoquinolines were examined for antiproliferative activity against the human cancer cell lines in the National Cancer Institute screen, in which the activity of each compound was evaluated with approximately 55 different cancer cell lines of diverse tumor origins. The GI50 values obtained with selected cell lines, along with the mean graph midpoint (MGM) values, are summarized in Table 1. The MGM is based on a calculation of the average GI50 for all of the cell lines tested (approximately 55) in which GI50 values below and above the test range (10^{-8} to 10^{-4} molar) are taken as the minimum (10^{-8} molar) and maximum (10^{-4} molar) drug concentrations used in the screening test. In addition, the relative activities of the compounds in the top1-mediated DNA cleavage assay are listed in Table 1. For comparison purposes, the activities of the previously reported lead compound **2**[2] and its more potent *N*-3'-aminoalkyl derivative **7**[6] are also included in the table.

Judging from the mean graph midpoints of the indenoisoquinoline-camptothecin hybrid molecules **8** (MGM 91.2 μM) and **13** (MGM 58.9 μM) relative to the lead compound **2** (MGM 20.0 μM), making the indenoisoquinolines more "camptothecin-like" was not a particularly good strategy for increasing their cytotoxicity in cancer cells, at least as evidenced by these two examples. Both compounds were generally less cytotoxic than the lead compound **2**. The hybrid molecule **13** and the lead compound **2** displayed similar potencies as top1 inhibitors. Interestingly, the pattern of top1-mediated DNA cleavage sites induced by **13** resembles more that of the lead compound **2** than that of camptothecin (data not shown). The only difference between camptothecin **(3)** and analogues **8** and **13** is the replacement of the lactone ring of

camptothecin (3) by either a dimethoxybenzene ring in 8 or a benzene ring in 13. A recently documented hydrogen bonding interaction between the hydroxyl group of the camptothecin analogue topotecan and the carboxyl group of Asp533 of the enzyme contributes to the binding of the camptothecin ring system, and an analogous interaction is not possible with 8 and 13, which may help to explain why these analogues are not as potent as camptothecin.[8] The importance of the hydroxyl group for camptothecin activity is also emphasized by the lack of activity displayed by 20-deoxycamptothecin (27) in a variety of biological systems.[15-18] In addition, the recently published X-ray crystallography studies show that the carboxylate and hydroxyl groups of the ring-opened hydroxyacid form of the lactone 28 of topotecan (29) also contribute to its binding in the ternary complex,[8] and these interactions would obviously not be possible with 8 and 13.

In order to investigate whether or not steric factors might play a role in the lower potency of 13, a model was constructed by overlapping the structure of the hybrid 13 with the structure of topotecan in the published ternary complex and then deleting the camptothecin structure (Figure 4).[8] The synthetic double-stranded DNA in the crystalline ternary complex contained a phosphorothiolate at the cleavage site.[8] As shown in Figure 4, the lower activity of 13, in comparison to camptothecin, as a top1 inhibitor does not appear to be due to steric factors, since it can be modeled into the camptothecin binding site in the ternary complex without any obvious steric constraints. This result re-emphasizes the importance of the hydroxylated lactone moiety in camptothecin (3), since the only difference in the structure of the hybrid 13 relative to 3 is the replacement of the substituted lactone ring of 3 with a benzene ring in 13.

Turning to the halogenated 11-alkenyl side chain derivatives 18-21 and 24, they were all approximately in the same range of cytotoxicity as the lead compound 2, although the analogues 18-20 were slightly less cytotoxic, while compounds 21 and 24 were slightly more cytotoxic than 2. The four-carbon alkenyl halides 19 and 24 were inactive as top1 inhibitors, while the five-carbon bromide 20 and the six-carbon bromide 21 showed increasing activity against top1. However, both 20 and 21 were less potent as top1 inhibitors than the lead compound 2.

The most potent compound in the present series of new indenoisoquinolines, both in terms of cytotoxicity and top1 inhibitory activity, was the 11-(3'-aminopropenyl) derivative **26**. The introduction of the 11-(3'-aminopropenyl) substituent into the lead compound **2** resulted in a decrease in the cytotoxicity mean graph midpoint (MGM) from 20 μM to 0.34 μM, indicating a large increase in overall cytotoxicity in cancer cell cultures. In addition, the resulting compound **26** was more potent as a top1 poison than **2**. These effects on activity are very close to what was previously observed upon replacement of the *N*-methyl group of the lead compound **2** with a 3'-aminopropyl substituent resulting in compound **7**.[6] In the present case, the 3'-aminopropenyl side chain is assumed to project into the minor groove, where it could possibly interact with Asp533 or Arg364, or with a stacked base residue of the DNA.[8] The enhanced cytotoxicities of the amino compounds **26** and **7** may also possibly be due to their altered solubility properties, facilitated cellular uptake, and/or the electrostatic attraction of the positively-charged ammonium cation to the negatively-charged DNA phosphodiester backbone prior to intercalation into the cleavage complex.

Experimental Section

Melting points were determined in capillary tubes and are uncorrected. Infrared spectra were obtained using $CHCl_3$ as the solvent unless otherwise specified. Except where noted, ^1H NMR spectra were obtained using $CDCl_3$ as solvent and TMS as internal standard. ^1H NMR spectra were determined at 300 MHz. Microanalyses were performed at the Purdue University Microanalysis Laboratory. Analytical thin-layer chromatography was carried out on Analtech silica gel GF 1000 micron glass plates. Compounds were visualized with short wavelength UV light. Silica gel flash chromatography was performed using 230-400 mesh silica gel.

8,9-Dimethoxy-12*H*-5,11a-diaza-dibenzo[*b,h*]fluoren-11-one (8). A solution of **9**[10] (200 mg, 0.58 mmol) in anhydrous THF (20 mL) was degassed by bubbling argon through the solution for 30 min. Liquid NH_3 was added via cold finger for 5 min at approximately 1 drop/5sec. The cold finger was removed and the reaction mixture was allowed to warm to room temperature under argon. The reaction mixture was stirred at room temperature for 12 h, at which point argon was bubbled through the solution for 1.5 h to remove excess NH_3. Anhydrous THF (10 mL) and NEt_3 (3 mL) were added and the reaction mixture was stirred at room temperature for 30 min. Bromide **11**[12] was added and the reaction mixture was stirred at room temperature for 24 h. The solvent was removed in vacuo and replaced with 10% NaOAc/AcOH (30 mL). The reaction mixture was stirred at room temperature for 24 h, at which point the solvent was removed in vacuo. The resulting solid was dissolved in water (100 mL) and extracted with $CHCl_3$ (3 × 100 mL). The organic layers were pooled, washed with saturated aqueous $NaHCO_3$ (1 × 100 mL), brine, dried ($MgSO_4$), filtered, and concentrated in vacuo to provide a brown solid. Purification (silica gel, $CHCl_3$) provided **8** (106 mg, 53%) as a yellow solid: ^1H NMR (300 MHz, DMSO-d_6) δ 8.61 (s, 1 H), 8.12 (m, 2 H), 7.84 (m, 1 H), 7.71 (s, 1 H), 7.82 (m, 1 H), 7.61 (s, 1 H), 7.50 (s, 1 H), 5.32 (s, 2 H), 3.94 (s, 3 H), 3.91 (s, 3 H); ESIMS *m/z* (rel intensity) 345.2 (100, MH^+). Anal. ($C_{21}H_{16}N_2O_3 \cdot 0.5H_2O$) C, H, N.

12*H*-5,11a-Diaza-dibenzo[*b,h*]fluoren-11-one (13). A solution of **9**[10] (200 mg, 0.58 mmol) in anhydrous THF (20 mL) was degassed by bubbling argon through the solution for 30

min. Liquid NH_3 was added via cold finger for 5 min at approximately 1 drop/5sec. The cold finger was removed and the reaction mixture was allowed to warm to room temperature under argon. The reaction mixture was stirred at room temperature for 12 h, at which point argon was bubbled through the solution for 1.5 h to remove excess NH_3, affording a solution of intermediate **10**. Anhydrous THF (10 mL) and NEt_3 (3 mL) were added and the reaction mixture was stirred at room temperature for 30 min. Chloride **12**[13] (195 mg, 1.16 mmol) was added and the reaction mixture stirred at room temperature for 24 h. The solvent was removed in vacuo and replaced with 10% NaOAc/AcOH (30 mL). The reaction mixture was stirred at room temperature for 24 h, at which point the solvent was removed in vacuo. The resulting solid was dissolved in water (100 mL) and extracted with $CHCl_3$ (3 × 100 mL). The organic layers were pooled, washed with saturated aqueous $NaHCO_3$ (1 × 100 mL), brine, dried ($MgSO_4$), filtered, and concentrated in vacuo to provide a brown solid. Purification (silica gel, $CHCl_3$) provided **13** (90 mg, 55%) as a yellow solid: IR (film) 3062, 2952, 2839, 1714, 1619, 1566, 1456, 1438, 1256, 1067 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.56 (d, J = 7.56 Hz, 1 H), 8.34 (s, 1 H), 8.23 (d, J = 8.64 Hz, 1 H), 7.91 (d, J = 8.19 Hz, 1 H), 7.90-7.71 (m, 3 H), 7.68 (s, 1 H), 7.66-7.56 (m, 2 H), 5.38 (d, J = 1.07 Hz, 2 H); ESIMS *m/z* (rel intensity) 285.2 (100, MH^+). Anal. ($C_{19}H_{12}N_2O \cdot 0.25H_2O$) C, H, N.

11-(4'-Chlorobutylidene)-5,6-dihydro-2,3-dimethoxy-6-methyl-8,9-methylenedioxy-5-oxo-11*H*-indeno[1,2-*c*]isoquinoline (18). $TiCl_4 \cdot 2THF$ (508 mg, 1.52 mmol), Zn dust (199 mg, 3.04 mmol), and dry THF (15 mL) were added to a flame-dried two-necked flask equipped with a magnetic stir bar and reflux condenser. The suspension was heated at reflux under argon for 3 h, after which a solution of 4-chlorobutanal (**14**)[19] (108.1 mg, 1.01 mmol) and indenoisoquinoline **2**[1] (185 mg, 0.51 mmol) in dry THF (15 mL) was introduced by syringe. The reaction mixture was heated at reflux for 2.5 h, after which 4 N HCl (20 mL) was added. The solution was stirred at room temperature for 1 h and then allowed to stand for 3 h. The resulting orange precipitate was collected by vacuum filtration. The solid was purified by flash chromatography (silica gel, 5:1 $CHCl_3$/hexanes) to provide **18** (96.1 mg, 43%) as an orange

solid: mp 196-201 °C; IR (film) 2926, 1636, 1610, 1517, 1483, 1255, 1034 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.89 (s, 1 H), 7.78 (s, 1 H), 7.40 (s, 1 H), 7.37 (s, 1 H), 6.88 (t, J = 7.19 Hz, 1 H), 6.04 (s, 2 H), 4.05 (s, 3 H), 4.03 (s, 3 H), 4.02 (s, 3 H), 3.74 (t, J = 6.32 Hz, 2 H), 3.00 (dt, J = 7.30 and 7.45 Hz, 2 H), 2.20 (qn, J = 6.90 Hz, 2 H); ESIMS *m/z* (rel intensity) 440.7 (100, MH^+), 442.6 (43, MH^+). Anal. ($C_{24}H_{22}ClNO_5$) C, H, N.

11-(4'-Bromobutylidene)-5,6-dihydro-2,3-dimethoxy-6-methyl-8,9-methylenedioxy-5-oxo-11*H*-indeno[1,2-*c*]isoquinoline (19). A 100 mL two-necked round-bottomed flask equipped with a magnetic stirring bar, reflux condenser, septa, and argon line was charged with zinc dust (537 mg, 8.21 mmol) and was flame dried. THF (30 mL) and a 1 M solution of $TiCl_4$ in toluene (4.11 mL, 4.11 mmol) were added. The suspension was heated at reflux for 5 h, at which point a suspension of **2**[1] (500 mg, 1.37 mmol) and 4-bromobutanal (**15**)[20,21] (413 mg, 2.74 mmol) in THF (30 mL) was added by pipette. The reaction mixture was heated at reflux for 1 h and then quenched with 4 N HCl (40 mL). The solution was stirred for 1 h and then cooled at 0 °C for 2 h. The orange precipitate was collected by vacuum filtration to provide an orange solid. This was purified by flash chromatography (silica gel, $CHCl_3$) to provide **19** (161.6 mg, 30%) as an orange solid: mp 197-199 °C; IR (film) 2921, 1610, 1517, 1483, 1381, 1296, 1253, 1207, 1033 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.88 (s, 1 H), 7.44 (s, 1 H), 7.40 (s, 1 H), 7.36 (s, 1 H), 6.83 (t, J = 7.11 Hz, 1 H), 6.05 (s, 2 H), 4.00 (s, 6 H), 3.97 (s, 3 H), 3.57 (t, J = 6.33 Hz, 2 H), 2.97 (q, J = 7.23 Hz, 2 H), 2.25 (qn, J = 6.80 Hz, 2 H); ESIMS *m/z* (rel intensity) 486.2 (97, MH^+), 484.2 (100, MH^+). Anal. ($C_{24}H_{22}BrNO_5$) C, H, N.

11-(5'-Bromopentylidene)-5,6-dihydro-2,3-dimethoxy-6-methyl-8,9-methylenedioxy-5-oxo-11*H*-indeno[1,2-*c*]isoquinoline (20). A 100 mL two-necked round-bottomed flask equipped with a reflux condenser and magnetic stir bar was charged with zinc dust (537 mg, 8.21 mmol) and flame dried. THF (30 mL) and 1 M $TiCl_4$ in toluene (4.11 mL, 4.11 mmol) were added to the round-bottomed flask and the mixture was heated at reflux for 6 h. THF (30 mL), 5-bromopentanal (**16**)[21] (452 mg, 2.74 mmol) and **2**[1] (500 mg 1.37 mmol) were added to the reaction mixture, which was heated at reflux for 2 h. The reaction mixture was cooled to room

temperature, 4 N HCl (40 mL) was added, and this mixture was stirred for 30 min and cooled in a –20 °C freezer overnight. The resulting yellow precipitate was collected by vacuum filtration and purified by flash chromatography to provide **20** (133.7 mg, 33%) as an orange solid: mp 196-197 °C; IR (film) 2 932, 1 632, 1 612, 1 517, 1483, 1 254, 1 032 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.89 (s, 1 H), 7.46 (s, 1 H), 7.41 (s, 1 H), 7.32 (s, 1 H), 6.86 (t, J = 6.83 Hz, 1 H), 6.05 (s, 2 H), 4.05 (s, 3 H), 4.03 (s, 3 H), 4.01 (s, 3 H), 3.49 (t, J = 6.47 Hz, 2 H), 2.86 (q, J = 7.15 Hz, 2 H), 2.06 (m, 2 H), 1.88 (m, 2 H); ESIMS *m/z* (rel intensity) 500.2 (95, MH^+), 498.2 (100, MH^+). Anal. ($C_{25}H_{24}BrNO_5$) C, H, N.

11-(6'-Bromohexylidene)-5,6-dihydro-2,3-dimethoxy-6-methyl-8,9-methylenedioxy-5-oxo-11*H*-indeno[1,2-*c*]isoquinoline (21). A 100 mL two-necked round-bottomed flask equipped with a reflux condenser and magnetic stir bar was charged with zinc dust (537 mg, 8.21 mmol) and flame dried. THF (30 mL) and 1 M $TiCl_4$ in toluene (4.11 mL, 4.11 mmol) were added to the round-bottomed flask and the mixture was heated at reflux for 4 h. Anhydrous THF (30 mL), 6-bromohexanal (**17**)[21] (491 mg, 2.74 mmol) and **2**[1] (500 mg 1.37 mmol) were added to the reaction mixture, which was heated at reflux for 2 h. The reaction mixture was cooled to room temperature, 4 N HCl (40 mL) was added, and this mixture was stirred for 30 min and then cooled in a –20 °C freezer overnight. The resulting yellow precipitate was collected by vacuum filtration and purified by flash chromatography to provide **21** (118.0 mg, 17%) as an orange solid: mp 182-185 °C; IR (film) 2929, 1636, 1610, 1516, 1482, 1296, 1255, 1033 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.88 (s, 1 H), 7.46 (s, 1 H), 7.40 (s, 1 H), 7.32 (s, 1 H), 6.88 (t, J = 6.96 Hz, 1 H), 6.05 (s, 2 H), 4.06 (s, 3 H), 4.02 (s, 3 H), 4.00 (s, 3 H), 3.44 (t, J = 6.62 Hz, 2 H), 2.84 (q, J = 7.13 Hz, 2 H), 1.95 (qn, J = 7.04 Hz, 2 H), 1.74 (m, 2 H) 1.66 (m, 2 H); ESIMS *m/z* (rel intensity) 514.2 (100, MH^+), 512.2 (91, MH^+). Anal. ($C_{26}H_{26}BrNO_5$) C, H, N.

11-(4'-Iodobutylidene)-5,6-dihydro-2,3-dimethoxy-6-methyl-8,9-methylenedioxy-5-oxo-11*H*-indeno[1,2-*c*]isoquinoline (24). NaI (217 mg, 1.45 mmol) was added to a suspension of bromide **19** (70 mg, 0.15 mmol) in acetone (15 mL). The reaction mixture was heated at reflux for 12 h, after which the resulting orange precipitate was collected by vacuum filtration and

purified by flash chromatography (silica gel, $CHCl_3$) to provide **24** (73.0 mg, 95%) as an orange solid: mp 173-174.5 °C; IR (KBr) 2944, 1612, 1522, 1481, 1254, 1033 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.88 (s, 1 H), 7.43 (s, 1 H), 7.40 (s, 1 H), 7.36 (s, 1 H), 6.84 (t, J = 7.22 Hz, 1 H), 6.05 (s, 2 H), 4.08 (s, 3 H), 4.04 (s, 3 H), 4.00 (s, 3 H), 3.35 (t, J = 6.69 Hz, 2 H), 2.95 (q, J = 7.32 Hz, 2 H), 2.19 (m, 2 H); ESIMS *m/z* (rel intensity) 532.1 (100, MH^+). Anal. ($C_{24}H_{22}INO_5$) C, H, N.

11-(3'-Aminopropylidene)-5,6-dihydro-2,3-dimethoxy-6-methyl-8,9-methylenedioxy-5-oxo-11*H*-indeno[1,2-*c*]isoquinoline (26). $TiCl_4$-THF (1:2) complex (730 mg, 2.19 mmol) and zinc dust (284 mg, 4.37 mmol) were put in a three-necked round-bottomed flask. THF (30 mL) was added. The resulting suspension was heated under reflux for 4 h. At this point, a mixture of aldehyde **25**[14] (189 mg, 1.09 mmol) and indenoisoquinoline **2** (266 mg, 0.73 mmol) in THF (30 mL) was added via syringe. The reaction mixture was stirred under reflux for an additional 4 h. Then 3 N HCl (10 mL) was added after cooling to room temperature and the mixture was stirred at room temperature for 1 h, followed by 0 °C for 2 h, and finally at room temperature overnight. The mixture was cooled to 0 °C and solid $NaHCO_3$ was added to neutralize HCl. The solvents were evaporated and the residue was subjected to flash chromatography, eluting with $CHCl_3$-MeOH (4:1) to provide **26** as a yellow powder (121 mg, 41%): mp >180 °C (dec); ^1H NMR (300 MHz, DMSO-d_6) 7.68 (s, 1 H), 7.62 (s, 1 H), 7.51 (s, 1 H), 7.48 (s, 1 H), 6.94 (t, J = 6.0 Hz, 1 H), 6.15 (s, 2 H), 4.01 (s, 3 H), 3.95 (s, 3 H), 3.87 (s, 3 H), 3.12-3.18 (m, 4 H). ESIMS *m/z* (rel intensity) 407.0 (100, MH^+). Anal. ($C_{23}H_{22}N_2O_5 \cdot 0.9CHCl_3$) C, H, N.

Top1-Mediated DNA Cleavage Reactions. Human recombinant top1 was purified from Baculovirus as described previously.[22] The 161 bp fragment from pBluescript SK(-) phagemid DNA (Stratagene, La Jolla, CA) was cleaved with the restriction endonuclease Pvu II and Hind III (New England Biolabs, Beverly, MA) in supplied NE buffer 2 (10 μL reactions) for 1 h at 37 °C, and separated by electrophoresis in a 1% agarose gel made in 1X TBE buffer. The 161 bp fragment was eluted from the gel slice (centrilutor by Amicon) and concentrated in a centricon

50 centrifugal concentrator (Amicon, Beverly, MA). Approximately 200 ng of the fragment was 3'-end labeled at the Hind III site by fill-in reaction with [alpha-^{32}P]-dGTP and 0.5 mM dATP, dCTP, and dTTP, in React 2 buffer (50 mM Tris-HCl, pH 8.0, 100 mM $MgCl_2$, 50 mM NaCl) with 0.5 units of DNA polymerase I (Klenow fragment). Unincorporated ^{32}P-dGTP was removed using mini Quick Spin DNA columns (Roche, Indianapolis, IN), and the eluate containing the 3'-end-labeled 161 bp fragment was collected. Aliquots (approximately 50,000 dpm/reaction) were incubated with top1 at 22 °C for 30 min in the presence of the tested drug. Reactions were terminated by adding SDS (0.5% final concentration).[2] The samples (10 μL) were mixed with 30 μL of loading buffer (80% formamide, 10 mM sodium hydroxide, 1 mM sodium EDTA, 0.1% xylene cyanol, and 0.1% bromophenol blue, pH 8.0). Aliquots were separated in denaturing gels (16% polyacrylamide, 7 M urea). Gels were dried and visualized by using a Phosphoimager and ImageQuant software (Molecular Dynamics, Sunnyvale, CA).

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Supporting Information Available: Combustion analyses for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Table 1. Cytotoxicities and Topoisomerase I Inhibitory Activities of Indenoisoquinoline Analogs.

	cytotoxicity (GI50 in μM)[a]									
compd	lung HOP-62	colon HCT-116	CNS SF-539	melanoma UACC-62	ovarian OVCAR-3	renal SN12C	prostate DU-145	breast MDA-MB-435	MGM[b]	Top 1 Cleavage[c]
8	>100	57.3	>100	>100	>100	>100	>100	>100	91.2	+
13	68.2	32.7	66.7	97.2	39.8	>100	>100	41.8	58.9	++
18	17.5	40.4	NT[d]	33.3	42.3	>100	35.9	>100	33.1	+
19	19.4	37.8	30.0	11.4	58.1	>100	67.8	>100	27.8	0
20	26.6	9.5	7.1	>100	>100	>100	4.5	>100	33.1	±
21	3.1	6.1	5.7	3.9	23.6	5.7	5.5	19.2	7.8	+
24	27.6	0.56	4.3	3.5	22.8	8.8	28.7	5.2	16.8	0
26	0.071	0.028	0.42	0.20	0.56	0.58	0.37	1.8	0.34	+++
2	1.3	35	41	4.2	73	68	37	96	20	++
7	0.06	0.13	0.26	0.25	0.31	0.31	0.04	1.21	0.16	+++

[a] The cytotoxicity GI50 values are the concentrations corresponding to 50% growth inhibition. [b] Mean graph midpoint for growth inhibition of all human cancer cell lines successfully tested. [c]The compounds were tested at concentrations ranging up to 10 μM. The activity of the compounds to produce top1-mediated DNA cleavage was expressed semi-quantitatively as follows: +: weak activity; ++: similar activity as the parent compound **2**; +++ & ++++: greater activity than the parent compound **2** ; ++++: similar activity as camptothecin (**3**).

Figure Legends

Figure 1. Electrostatic potential surfaces for indenoisoquinoline **2** (top), camptothecin **3** (middle), and the hybrid structure **8** (bottom). The values are color coded onto the total electron density surface, with colors toward red indicating electron-rich regions of the molecule.

Figure 2. Hypothetical model of the orientation of indenoisoquinoline **7** relative to DNA in the ternary complex containing topl, DNA, and the inhibitor **7**.

Figure 3. ^{1}H NMR Spectrum of indenoisoquinoline **19**.

Figure 4. Model of the binding of the camptothecin-indenoisoquinoline hybrid **13** in the ternary complex consisting of DNA, topl, and the inhibitor. The diagram is programmed for wall-eyed viewing.

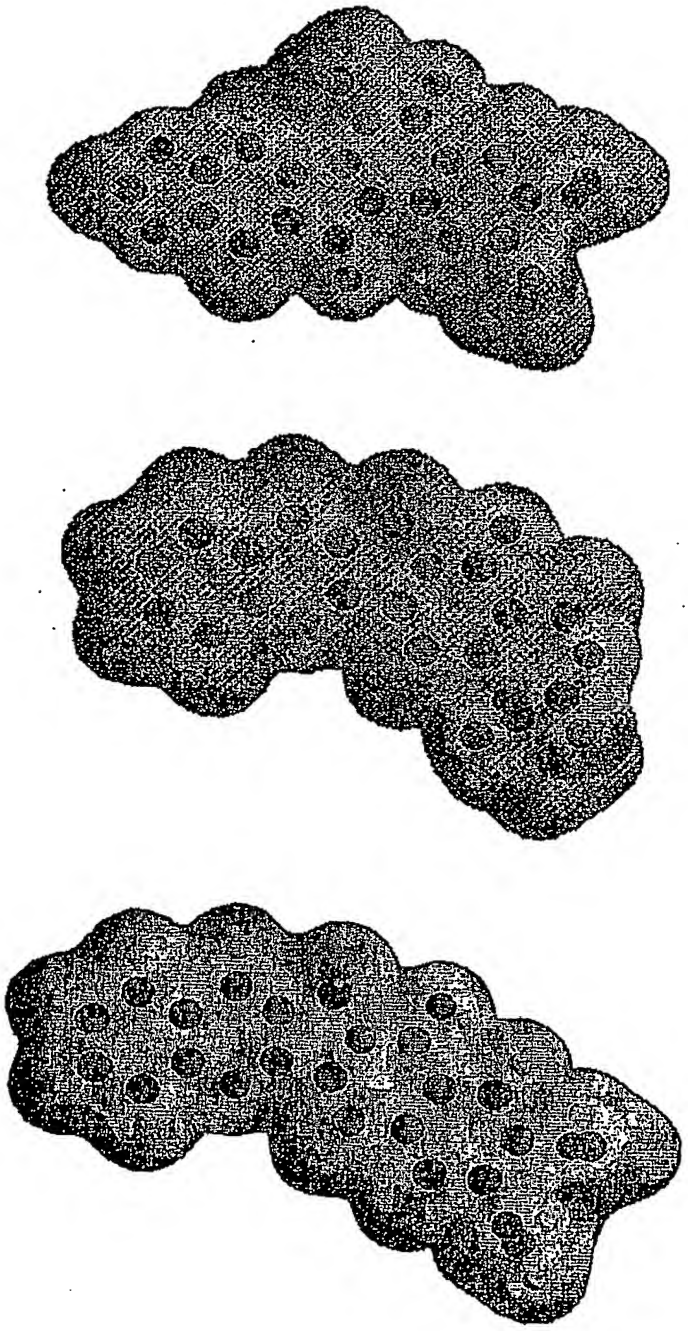


Figure 1. Electrostatic potential surfaces for indenoisoquinoline **2** (top), camptothecin **3** (middle), and the hybrid structure **8** (bottom). The values are color coded onto the total electron density surface, with colors toward red indicating electron-rich regions of the molecule.

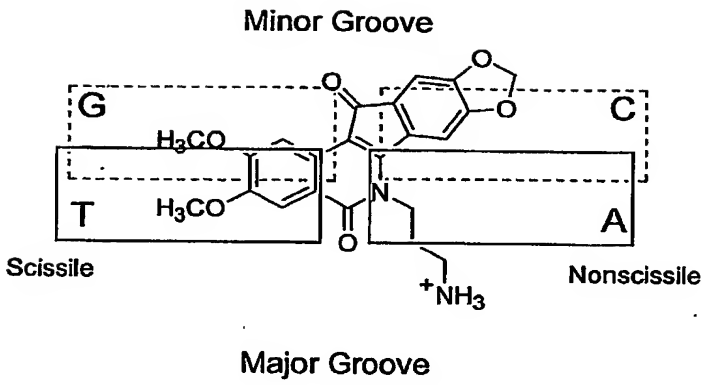


Figure 2. Hypothetical model of the orientation of indenoisoquinoline **7** relative to DNA in the ternary complex containing top1, DNA, and the inhibitor **7**.

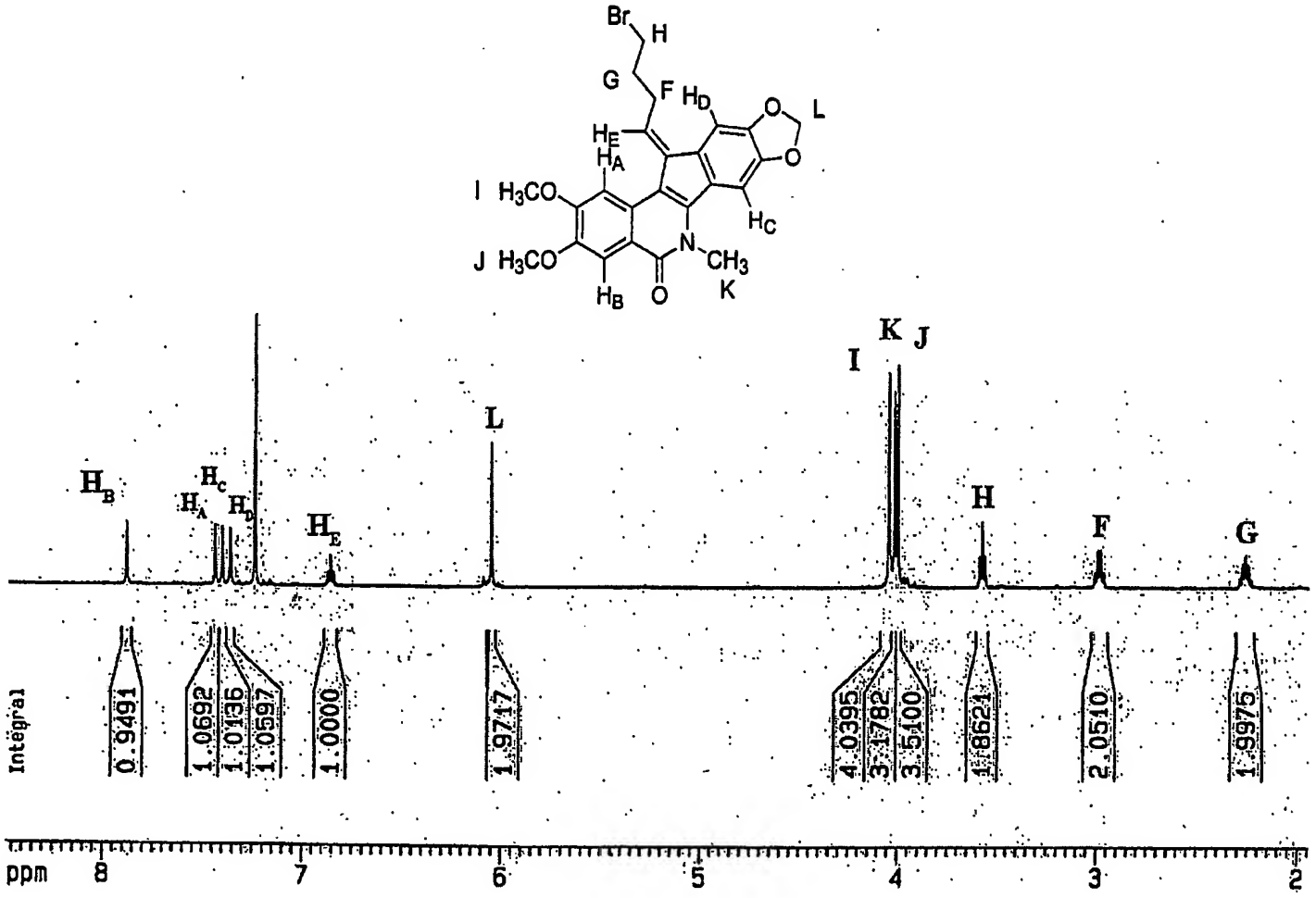


Figure 3. ^{1}H NMR Spectrum of indenoisoquinoline 19.

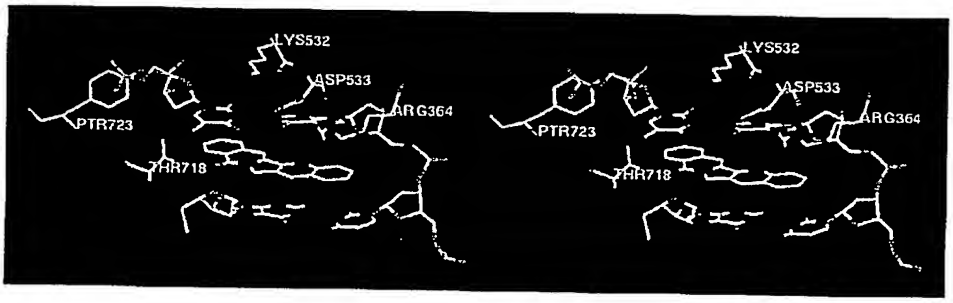
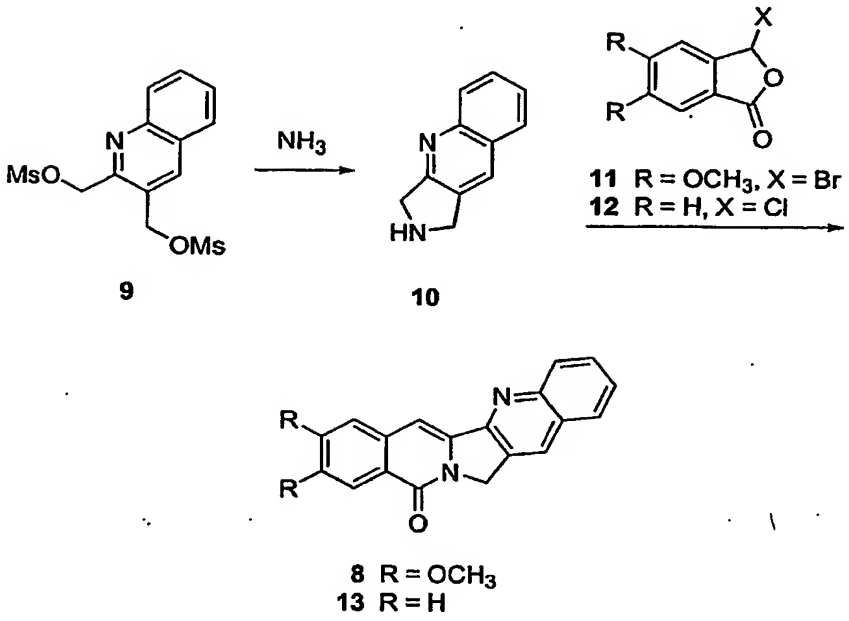


Figure 4. Model of the binding of the camptothecin-indenoisoquinoline hybrid **13** in the ternary complex consisting of DNA, top1, and the inhibitor. The diagram is programmed for wall-eyed viewing.

Scheme 1



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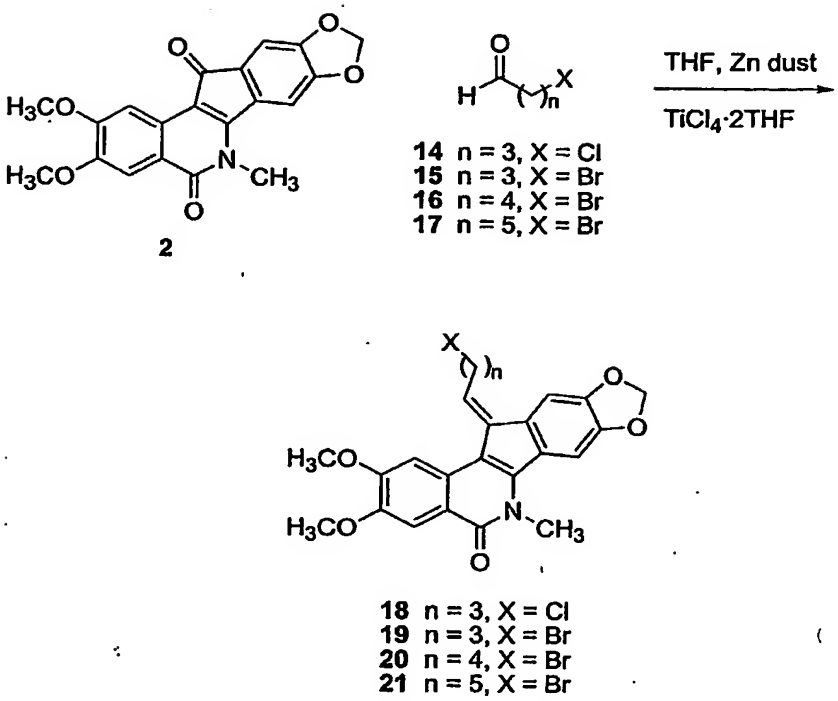
Scheme 2



2

14 n = 3, X = Cl
15 n = 3, X = Br
16 n = 4, X = Br
17 n = 5, X = Br

18 n = 3, X = Cl
19 n = 3, X = Br
20 n = 4, X = Br
21 n = 5, X = Br

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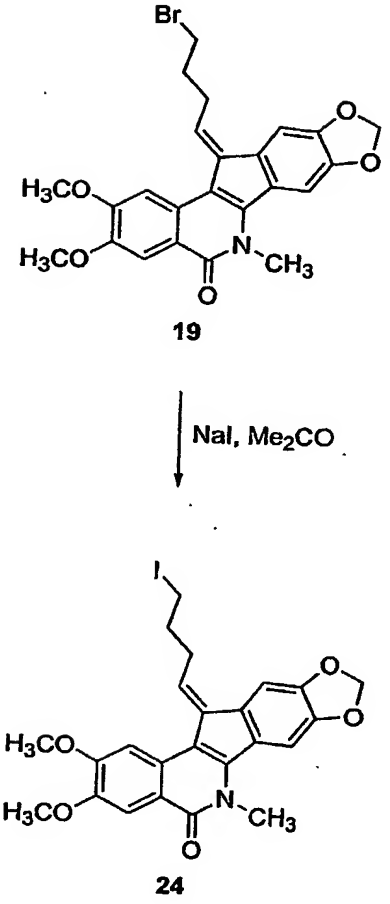
Scheme 3



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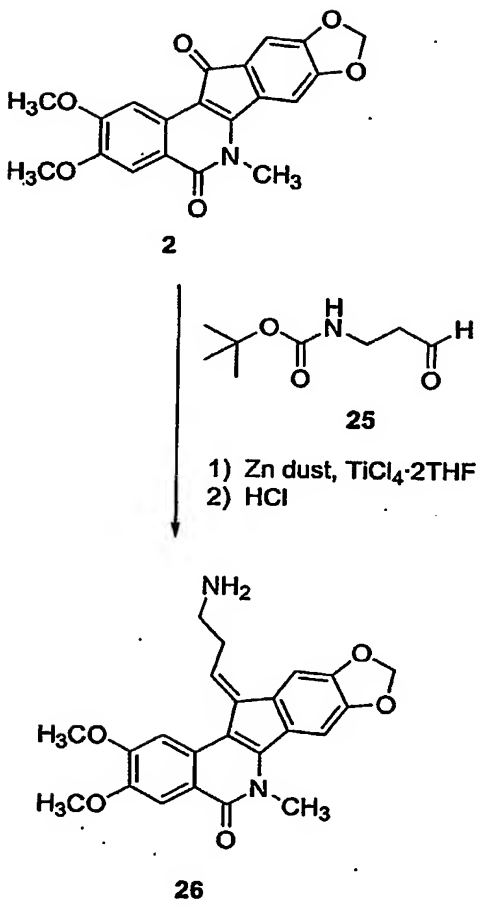
Scheme 4



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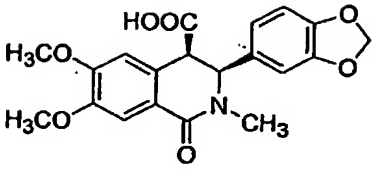

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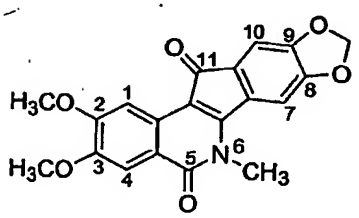

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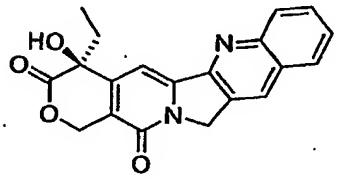

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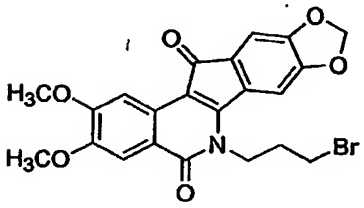

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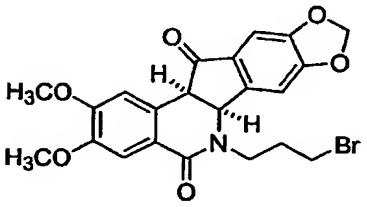

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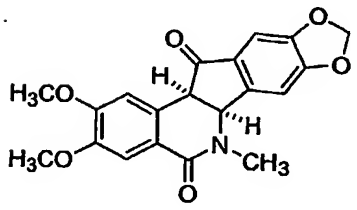

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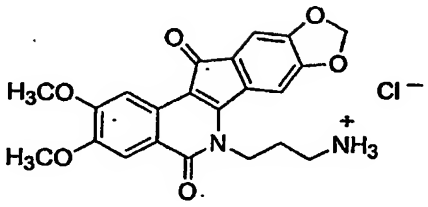

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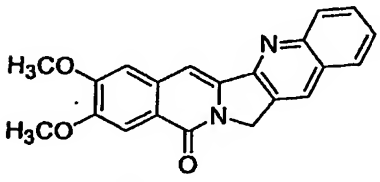

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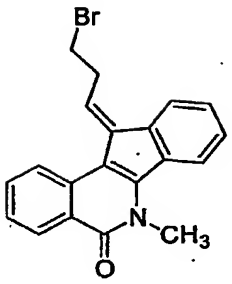

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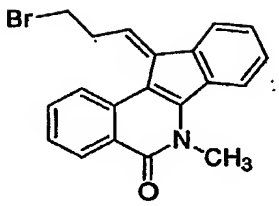

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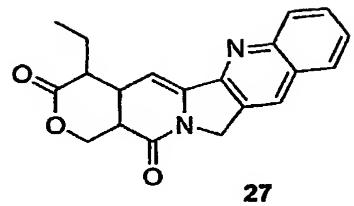

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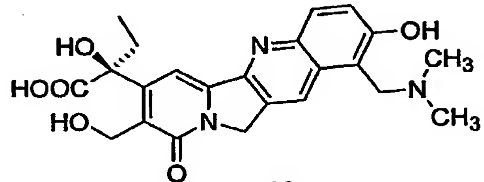
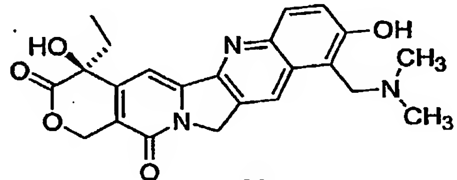


28



29

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Supporting Information

Design, Synthesis, and Biological Evaluation of Cytotoxic 11-Alkenylindenoisoquinoline Topoisomerase I Inhibitors and Indenoisoquinoline-Camptothecin Hybrids

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Elemental Analyses

8,9-Dimethoxy-12*H*-5,11a-diaza-dibenzo[*b*,*h*]fluoren-11-one (8). Anal. Calcd for $C_{21}H_{16}N_2O_3 \cdot 0.5H_2O$: C, 71.38; H, 4.85; N, 7.93. Found: C, 71.07; H, 4.72; N, 7.85.

12*H*-5,11a-Diaza-dibenzo[*b*,*h*]fluoren-11-one (13). Anal. Calcd for $C_{19}H_{12}N_2O \cdot 0.25H_2O$: C, 79.01; H, 5.46; N, 9.70. Found: C, 79.30; H, 5.36; N, 9.66.

11-(4'-Chlorobutylidene)-5,6-dihydro-2,3-dimethoxy-6-methyl-8,9-methylenedioxy-5-oxo-11*H*-indeno[1,2-*c*]isoquinoline (18). Anal. Calcd for $C_{24}H_{22}ClNO_5$: C, 65.53; H, 5.04; N, 3.18. Found: C, 65.30; H, 4.96; N, 3.08.

11-(4'-Bromobutylidene)-5,6-dihydro-2,3-dimethoxy-6-methyl-8,9-methylenedioxy-5-oxo-11*H*-indeno[1,2-*c*]isoquinoline (19). Anal. Calcd for $C_{24}H_{22}BrNO_5$: C, 59.52; H, 4.58; N, 2.89. Found: C, 59.56; H, 4.56; N, 2.88.

11-(5'-Bromopentylidene)-5,6-dihydro-2,3-dimethoxy-6-methyl-8,9-methylenedioxy-5-oxo-11*H*-indeno[1,2-*c*]isoquinoline (20). Anal. Calcd for $C_{25}H_{24}BrNO_5$: C, 60.25; H, 4.85; N, 2.81. Found: C, 60.57; H, 4.90; N, 2.83.

11-(6'-Bromohexylidene)-5,6-dihydro-2,3-dimethoxy-6-methyl-8,9-methylenedioxy-5-oxo-11*H*-indeno[1,2-*c*]isoquinoline (21). Anal. Calcd for $C_{26}H_{26}BrNO_5$: C, 60.95; H, 5.11; N, 2.73. Found: C, 60.55; H, 5.08; N, 2.72.

11-(4'-Iodobutylidene)-5,6-dihydro-2,3-dimethoxy-6-methyl-8,9-methylenedioxy-5-oxo-11*H*-indeno[1,2-*c*]isoquinoline (24). Anal. Calcd for $C_{24}H_{22}INO_5$: C, 54.25; H, 4.17; N, 2.64. Found: C, 54.64; H, 4.25; N, 2.60.

11-(3'-Aminopropylidene)-5,6-dihydro-2,3-dimethoxy-6-methyl-8,9-methylenedioxy-5-oxo-11*H*-indeno[1,2-*c*]isoquinoline (26). Anal. Calcd for $C_{23}H_{22}N_2O_5 \cdot 0.9CHCl_3$: C, 55.86; H, 4.49; N, 5.45. Found: C, 55.86; H, 4.72; N, 5.24.